REMARKS

The Office Action has been carefully studied. No claim is allowed. Claims 1, 7-10, 12-16, 25, 28, 38, 39, 41, 42, 46, 48-50, 52, and 56-60 presently appear in this application and define patentable subject matter warranting their allowance. Reconsideration and allowance are hereby respectfully solicited.

The face-to-face interview among Dr. Wu, one of the present inventors, and the undersigned, representing applicants, and Examiners Dr. Kaushal and Dr. Fredman on August 3, 2004, is hereby gratefully acknowledged. Applicants' representatives wish to thank the examiners for the courtesies extended during this interview.

No specific prior art was discussed at the interview as there were no prior art issues raised in the Office Action. All the claims however were discussed, and applicants' representatives agreed to cancel the claims directed to a DNA molecule or convert them to method claims. Applicants' representatives further presented evidence on the interchangeability of uromodulin promoters among mammalian species and also proposed to limit the claims to "mammalian uromodulin promoters", to delete the recitation of "ancestors" from the claims to transgenic non-human mammals, and to incorporate the recitation of expression of the heterologous polypeptide "to the ascending limb of Henle's loop and to the early distal tubules of the kidneys" in method claim 38 in order to overcome the enablement rejection. Agreement was reached that the enablement rejection would likely fall.

The written description rejection was also discussed at the interview, with applicants' representatives arguing that there is a

sufficient representative number of uromodulin promoter sequences identified in the instant specification and in the prior art to satisfy the written description requirements. The only agreement reached on this written description issue was that applicants would introduce new claims directed to specific mammalian uromodulin promoters and specific transgenic non-human mammals and the examiners would carefully consider this issue in view of applicants' arguments in the instant amendment. The argument presented at the interview as it relates to the written description rejection is incorporated below.

Claims 41 and 55 have been rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement (new matter issues).

The recitation of "pig... uromodulin promoter" is now deleted from claim 41, thereby obviating this part of the rejection. Claim 41 is now amended to recite "human... uromodulin promoter" in place of pig uromodulin promoter as supported on page 19, lines 15-27 of the specification.

The feature of "mammalian uromodulin promoter directs expression of said heterologous polypeptide *in vivo* to the ascending limb of Henle's loop and the early distal tubules of the kidneys" as now recited in claims 38, 57 and 59 (claim 55 is now cancelled) is supported on page 14, lines 23-26 and page 16, lines 21-27.

Reconsideration and withdrawal of this rejection are therefore respectfully requested.

Claim 8 has been rejected under 35 U.S.C. §112, second paragraph, as being indefinite for the recitation of "predicted β-

turns". This rejection appears to no longer be an issue as the examiners have accepted applicants' argument that predicting the location of β-turns using common and publicly available algorithm, such as the Chou-Fasman algorithms, among others, is well recognized by those of skill in the art and therefore is not indefinite even if the specific algorithm is not mentioned by name in the specification.

Claims 1, 7-10, 12-16, 29, 31-37, 47 and 55 have been rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. Claims 25, 28, 38-42, 44, 46, and 48-54 have also been separately rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. Both of these rejections are obviated by the amendments to the claims as discussed at the face-to-face interview on August 3, 2004, the evidence on interchangeability of mammalian promoters as presented below in applicants' arguments rebutting the written description rejection, and the merely routine experimentation that is required by those of skill in the art to obtain transgenic non-human mammals.

Claims 1, 7-10, 12-16, 25, 28-29, 31-42, 44, and 46-55 have been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This rejection is respectfully traversed.

The rejection as it relates to the scope of the transgenic animals and as discussed at the interview no longer appears to be at issue, particularly in view of the species of mammals recited in the claims being well described in the specification and further in view of

the examiner's agreement that enablement with regard to the different species of transgenic non-human mammals would be withdrawn as a result of the amendments to the claims.

The examiner holds that, with regard to uromodulin promoters, the instant specification only teaches the murine, goat and bovine uromodulin promoters but fails to disclose uromodulin promoter sequences and states, "Beside the mouse uromodulin promoter comprising the nucleotide sequences of SEQ ID NO:1, the specification as filed fails to disclose any other uromodulin promoter or fragment thereof (obtained from any amphibian, reptile, bird or all mammal) that directs the expression of a polypeptide *in vivo* in the kidney to produce the polypeptide in the urine."

In response to the examiner's position in this part of the rejection, applicants have now amended the claims to recite for only "mammalian" uromodulin promoters. As disclosed in the present specification at page 19, lines 15-18, the sequence of the mouse and goat uromodulin promoters have now been determined and reported by the applicants in the instant specification and the bovine, rat and human promoter regions have been previously reported in the prior art. On page 18, lines 15-19, a Yu et al. (1994) reference is cited for its disclosure of the bovine and rat (and human) uromodulin promoter regions and is furthermore incorporated by reference. This Yu et al. (1994) reference is the Yu et al., "Bovine and rodent Tamm-Horsfall protein (THP) genes-cloning, structural analysis, and promoter identification", Gene Expression 4:63-75 (1994) reference AM submitted with the Information Disclosure Statement dated July 13, 2000. For

clarification, uromodulin is also known as Tamm-Horsfall protein (THP). Fig. 5 in this Yu et al. (1994) reference shows a sequence alignment of the promoter regions of bovine, rat and human uromodulin. Accordingly, the uromodulin promoters from mouse, goat, cattle, rat, and human are disclosed in the instant specification and provides a representative number of species of the genus of mammalian uromodulin promoters so as to reasonably convey to those of skill in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. This is further supported by the following discussion below.

Zbiskowska et al. (2002), cited by the examiner, discloses that a human uromodulin promoter provides kidney-specific expression in transgenic mice that leads to production of a recombinant biologically active human antitrypsin in the urine. Zbiskowska arbitrarily chose a human uromodulin promoter region that also contains exon 1 and part of exon 2 to drive expression, even though the exon sequences are unnecessary for kidney-specific expression. This can be seen from applicants' own evidence (Zhu et al., 2002 and 2003, copies of which were submitted with the amendment filed July 28, 2003), where sequences from only the 5'-upstream non-coding region were found to be sufficient for directing high level kidney-specific expression.

The Kim et al., Transgenic Res. 12:191-201 (2003) reference, which was also submitted with the amendment of July 28, 2003, demonstrates that the most critical cis elements of the uromodulin gene are located within the 600 bp upstream region (see abstract), providing evidentiary support for applicants' expectation in the specification at

page 18, lines 23-28, that the approximately 600 base pairs of sequence upstream of the uromodulin coding sequence is sufficient for directing kidney specific expression. Moreover, like Zbiskowska's showing of a human uromodulin promoter being capable of kidney specific expression in mice, Kim et al. also establish that a bovine uromodulin promoter can likewise function in mice. These results demonstrate the interchangeability of mammalian uromodulin promoters from one mammalian species to another, which is altogether expected given the disclosure in the specification at page 17, lines 13-15, that uromodulin promoter sequences are likely to be conserved among mammals and the sequence conservation actually observed among the disclosed bovine, rat, human, mouse and goat uromodulin promoter sequences.

To extend the alignment for bovine, rat and human uromodulin promoter sequences in Fig. 5 of Yu et al. (1994), applicants have prepared a comparison/alignment of about 600 base pairs upstream of the uromodulin coding sequence from all five bovine, rat, human, mouse and goat uromodulin promoters. The first alignment attached hereto for the examiner's consideration is entitled "Highly conserved nature of the proximal promoter sequences of rat, mouse, cattle, goat and human uromodulin (Tamm-Horsfall protein)" and was generated using a PileUp program of an online sequence analysis software (SeqWeb). A consensus sequence, shown as the bottom line in the alignment, is readily generated from the alignment of uromodulin promoter sequences from the five mammalian species. The second sequence alignment attached hereto shows the conserved transcription factor binding sites in the proximal promoters of uromodulin.

Also attached hereto are computer printouts of sequence identity calculations (BestFit) between the approximately 600 bp uromodulin promoter sequences presented in the first and second sequence alignments. A table summarizing the sequence identity calculated between each of five uromodulin promoter sequences is provided as a further attachment for the examiner's consideration. This table shows that the level of sequence identity is exceedingly high for promoters, ranging from 73% to 94% over approximately 600 base pairs.

On page 14, lines 17-20 of the instant specification, it is disclosed that uromodulin (Tamm-Horsfall protein) is by far the most abundant urinary protein of human and higher mammals, with an excretion rate of up to 200 mg per day.

In conclusion, when those of skill in the art take the five disclosed mammalian uromodulin promoters altogether into consideration along with (1) the high sequence identity between the five identified uromodulin promoters (sufficient to generate a consensus sequence), (2) the expectation that other mammalian promoters would have similar high sequence identity to the five identified mammalian uromodulin promoters and the consensus mammalian uromodulin promoter, (3) the functional interchangeability of the mammalian promoters among mammalian species, and (4) the ease in readily identifying a uromodulin promoter based not only on its sequence (i.e., sequence identity) but also on its ability to drive the expression of the most abundant (and therefore easily detectable) urinary protein in mammals for secretion into the urine at the ascending limb of Henle's loop and the early distal tubules of the

kidneys, these same persons of skill in the art would immediately recognize that the inventors, at the time the application was filed, had possession of the claimed invention.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

In view of the above, the claims comply with 35 U.S.C. §112 and define patentable subject matter warranting their allowance. Favorable consideration and early allowance are earnestly urged.

Respectfully submitted,

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HIGHLY CONSERVED NATURE OF THE PROXIMAL PROMOTER SEQUENCES OF RAT, MOUSE, CATTLE, GOAT AND HUMAN UROMODULIN (TAMM-HORSFALL PROTEIN)

```
                                1                                  31
RAT                             C TAGTCTTGT. CTGACAGAGG TCCAGTTGAG
MOUSE                           C CAGTCTTGT. CTGACAGAGG TCCAGTTCAG
CATTLE                          C TGGTCCAATG ATGTCTGAAT TGCCTTCTGT
GOAT                            C TGGTCCAATG ATGTCTGAAT TATCTGCTGT
HUMAN                           C AGGTCCAGTG ATGTCTGAAC TACCTTCTGG
CONSENSUS                       C TGGTCCAGTG ATGTCTGAA- T-CCTTCTGG

                32                                                 81
RAT             GGATGTCCAG ATGGTCTTGC AACC.GATAA CTTTCTCAGA GACTCTCTCT
MOUSE           GAGTGTCCAG ATGGTCTGAT AACCTGATGC CATTCTCAGA GA....CTCT
CATTLE          CTCTGACCTT CAGGCATTCT CAGCTCCTTT CCTGCTCACA TCGGGACCCC
GOAT            CTCTGACCTT CAGCCATTCT CAGCTCCTTT CCTGATCACA TTGGGACCCC
HUMAN           TTCTGACTTT CAGCCATTCT CAGCTCCTCT CTTGCTTGTG TCTGGATTCT
CONSENSUS       -TCTGACCTT CAGGCATTCT CAGCTCCTTT C-TGCTCA-A T-GGGACTCT

                82                                                131
RAT             TTCCTGTCTG GACTCTAGTG GGGAGGACTA ATCTGGTGAA GCTGTTCTTC
MOUSE           TTCCTGTCTG GAATCTAGTG AGGAGGACTT ATCTGGTGAA GCTGTCCTTT
CATTLE          AGGGAAGCTG GTTGAACCCA TGAGGATGGA ACTTGCTTTG GAACTGAGTG
GOAT            AGGGGAGCTG GCTGAATCTG TGAGGATGGC ATTTGCTTTG GAATTAAGTG
HUMAN           AAGGCTGATC TCATGAGAAT GGGTGTTTCA GAAGGGTGCC CTCTCCA...
CONSENSUS       A-GGT-GCTG G--T-AA-TG -GG-G-T--A AT-TGGTG-- G---TCA-TG

                132                                               181
RAT             AGATCAGGTG TGTGTTCCAG GCTTCGAAGC AAATGTTTCT GTTATCCTAA
MOUSE           AGAACAGGAG TGTGTTCCAG TCTTCAAAGC AAACATTCCT TTTATCCTAA
CATTLE          GCCACAAGTA TACATCCCAG TGGGGACAGT GAGCACCCCT TTTCTCCTGG
GOAT            GCCACAAGTA CACATCCTGG TGGGGACGAT GAGCACCCCT TTTCTCCTGG
HUMAN           .AGACAGGTG CACCTCCCAT CTGGGGCAGT GAATA.TCCT TTTGTCCTTA
CONSENSUS       ---ACAGGTG TAC-TCCCAG T-GGGACAGT GAACA-TCCT TTT-TCCT-A

                182                                               231
RAT             CCCAGGCTGG CTTCAGATAT ..TGTCTTTT TTCCTGCCCC TTTGG..TAT
MOUSE           CACAGTCTGA CTTCAGATAT ACTGTCTTTT TCCTGGCTCC TTGGGCTTAG
CATTLE          AGCAGCCTGG CTTCAGATT. .CTGGCCTCT GCTT...T.. ..........
GOAT            AGCAGCCTGG CTTCAGATT. .CTGGCCTCT GCTTGGCT.. ..........
HUMAN           TGCAGCCTGG CTTCAGATA. .CTGGCTTCT GCCTGGCTCC TTG......A
CONSENSUS       -GCAGCCTGG CTTCAGATAT -CTGGCTTCT GCCTGGCTCC TTGGG--TA-

                232                                               281
RAT             TTCCACCTTG TCCTTGCCCA GGTCCAAGAA AAAGCCCAGA ACCTTGGCAC
MOUSE           GTCTACCTTG TCCTTGCCCA GGTCCAAGAA AAGGCCCAGA ACCTTGGCAC
CATTLE          ..CCACTTTG TGCTTTTCAA TGACCAAGAA .AAGCCCAGG CACTTGGAAT
GOAT            ..CCACTTTG TGCTTTTCAA TGACCAAGAA .AATCCCAGG CCCTTGGAAT
HUMAN           TCCCACCCTG CCCTTGTCAG TGACCAAGAA GAAGCCCAGC ACCTTGGCAC
CONSENSUS       TTCCACCTTG TCCTTGTCAA TGACCAAGAA AAAGCCCAG- ACCTTGGCAC
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                282                                                     331
RAT             TGCTTTGCCA GTTAATGTCT AACCGAGGAA TGTCTTGCTG CCAAAAGGTG
MOUSE           TGTTTTGCCA GTTAATGTCT AACTGAGGAA TGTCTTGCTG CCAAAAGGTG
CATTLE          TTTTTACCCA GTTAATTTCT AACTAAAGAA CCTCTCGTTG CCAAAAGATA
GOAT            TGTTTACTCA GTTAATTTCT AACTAAAGAA CCTCTTGTTG CCAAAAGGTA
HUMAN           TGCTTTCCCA GTTAATTTCT AACTATGGAA TCTCTTGCTG TTAGAAGGTG
CONSENSUS       TGTTTTCCCA GTTAATTTCT AACTAAGGAA TCTCTTGCTG CCAAAAGGTG

                332                                                     381
RAT             .CAAACAGAG ACCTTGTATT TCCAGGCACA GGTGTGACCC C CAATGTCA
MOUSE           .AAAACAGAG ACCTTGTATT TCCAGGCACA GGTGTGACCC C  AATGTCA
CATTLE          TAAAACAGAG CCCTTGTAAC TCTGGGCACA ACTGTGACCC C  AGTGTCA
GOAT            TAAAACAGAG CCCTTGTAGC TGTGGGCACA GCTGTGACCC C  CATGTCA
HUMAN           CGAAACAGTG ACCTTGTATT TCCGGGCACA GGTGTGACCC CCCAATGTCA
CONSENSUS       TAAAACAGAG ACCTTGTATT TCCGGGCACA GGTGTGACCC C-CAATGTCA

                382                                                     431
RAT             ATCATTTTCT GTCTCTAACT ACCAGAGGAA AAACTAACAA CAACAGCCTC
MOUSE           ATCATTT..T GTGTCTAACT CCCAGGGGAA AAACTAACAA CAACAGACTC
CATTLE          ATCATTTGGG GTCTCTACCT ATTAG GGAA AA  GAACAA CAACCACCTC
GOAT            ATCATTTGGG GTCTCTACCT ATTAG GGAA AA  GAACAA CANCCACCTC
HUMAN           ATCATTTGGG GTCTCTAGCT ATTA  GGAA AA AGAACAA CAACAACCTC
CONSENSUS       ATCATTTGGG GTCTCTA-CT ATTAG-GGAA AAAAGAACAA CAACAACCTC

                432                                                     481
RAT             ATGGTTTGGA AAAGGTGAAC TCTATGCCAA ATGGGAAGAA AAATTCTGAC
MOUSE           ATGGCTTGGA AAAGGTGAAT TCTATGCCAA AAGGGAAGGA AAGTTCT.AC
CATTLE          ACAGCCTAGA AAAGGAAAAC ACTGTGTCAA AAGGGAAAAA TATTCC..AC
GOAT            ACAGCCTANA AAAGGAAAAC ACTGTGTCAA AAGGGAAAAA TATTCC..AC
HUMAN           ACAGCTTGGA CAAGGCAAAC ATTATGCCAG GAGGAAAAAA TATTCC..AC
CONSENSUS       ACAGCTTGGA AAAGG-AAAC ACTATGCCAA AAGGGAAAAA TATTCCT-AC

                482                                                     531
RAT             CCCCACAGAA ACAATCTCAA GAGGCAGAAG CAGAGAATAA TTGGAGG.GA
MOUSE           CCCCACAGAA ACAATCTCAG AGGGCAGAAG CAGAGAATAA TCTGAGG.GA
CATTLE          CCCCATTAAA ATA..ATTAA GAAACAGAAC CAGAGGATCA TTGGAGGAAA
GOAT            CCCCATTAAA ATA..ATTAA GAAACAGAAC CAGAGGATCA TTGGAGGAGA
HUMAN           CCCCAAGAAA ACAATATCAA AAAACAGAAC TAGAGACTAA TTGGAGGAGA
CONSENSUS       CCCCA--AAA ACAATATCAA GAAACAGAAC CAGAGAATAA TTGGAGGAGA

                532                                                     581
RAT             GAGGGCCAGC CAAGGGCAGA CATATATATA TATATATTGA TCACAGGCAC
MOUSE           GAGGGCCAGC CAAGGGCAGG CAAGTATATA       TTGA TCACAGGCAC
CATTLE          GACTGCCAGT GGGGGACAGA TGTATATATA TAGATATGAT AGTCACCTAC
GOAT            GATTGCCAGT GGGGGACAGA TGTATATATA TAGATATGAA AGTCACCTAC
HUMAN           GATTGCCAGC CTGGGGCAAA TGTGTATATA TAAGTATGAG GCACA.....
CONSENSUS       GA-TGCCAGC C-GGGGCAGA TGTATATATA TA-ATATGAA -CACA---AC

                582     591
RAT             TTACTTGTGA
MOUSE           TTACTTGTGA
CATTLE          TTGTAAAAGG
GOAT            TTGTAAAAGG
HUMAN           TCATCACCAG
CONSENSUS       TTAT-A--GG
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CONSERVED TRANSCRIPTION FACTOR BINDING SITES IN THE PROMIXMAL PROMOTERS OF UROMODULIN

```
                                1                                  31
RAT                             C TAGTCTTGT. CTGACAGAGG TCCAGTTGAG
MOUSE                           C CAGTCTTGT. CTGACAGAGG TCCAGTTCAG
CATTLE                          C TGGTCCAATG ATGTCTGAAT TGCCTTCTGT
GOAT                            C TGGTCCAATG ATGTCTGAAT TATCTGCTGT
HUMAN                           C AGGTCCAGTG ATGTCTGAAC TACCTTCTGG
CONSENSUS                       C TGGTCCAGTG ATGTCTGAA- T-CCTTCTGG

                32                                                 81
RAT             GGATGTCCAG ATGGTCTTGC AACC.GATAA CTTTCTCAGA GACTCTCTCT
MOUSE           GAGTGTCCAG ATGGTCTGAT AACCTGATGC CATTCTCAGA GA....CTCT
CATTLE          CTCTGACCTT CAGGCATTCT CAGCTCCTTT CCTGCTCACA TCGGGACCCC
GOAT            CTCTGACCTT CAGCCATTCT CAGCTCCTTT CCTGATCACA TTGGGACCCC
HUMAN           TTCTGACTTT CAGCCATTCT CAGCTCCTCT CTTGCTTGTG TCTGGATTCT
CONSENSUS       -TCTGACCTT CAGGCATTCT CAGCTCCTTT C-TGCTCA-A T-GGGACTCT

                82                                                 131
RAT             TTCCTGTCTG GACTCTAGTG GGGAGGACTA ATCTGGTGAA GCTGTTCTTC
MOUSE           TTCCTGTCTG GAATCTAGTG AGGAGGACTT ATCTGGTGAA GCTGTCCTTT
CATTLE          AGGGAAGCTG GTTGAACCCA TGAGGATGGA ACTTGCTTTG GAACTGAGTG
GOAT            AGGGGAGCTG GCTGAATCTG TGAGGATGGC ATTTGCTTTG GAATTAAGTG
HUMAN           AAGGCTGATC TCATGAGAAT GGGTGTTTCA GAAGGGTGCC CTCTCCA...
CONSENSUS       A-GGT-GCTG G--T-AA-TG -GG-G-T--A AT-TGGTG-- G---TCA-TG

                132                                    TCF-1       181
RAT             AGATCAGGTG TGTGTTCCAG GCTTCGAAGC AAATGTTTCT GTTATCCTAA
MOUSE           AGAACAGGAG TGTGTTCCAG TCTTCAAAGC AAACATTCCT TTTATCCTAA
CATTLE          GCCACAAGTA TACATCCCAG TGGGGACAGT GAGCACCCCT TTTCTCCTGG
GOAT            GCCACAAGTA CACATCCTGG TGGGGACGAT GAGCACCCCT TTTCTCCTGG
HUMAN           .AGACAGGTG CACCTCCCAT CTGGGGCAGT GAATA.TCCT TTTGTCCTTA
CONSENSUS       ---ACAGGTG TAC-TCCCAG T-GGGACAGT GAACA-TCCT TTT-TCCT-A

                182          NF-GMB                                231
RAT             CCCAGGCTGG CTTCAGATAT ..TGTCTTTT TTCCTGCCCC TTTGG..TAT
MOUSE           CACAGTCTGA CTTCAGATAT ACTGTCTTTT TCCTGGCTCC TTGGGCTTAG
CATTLE          AGCAGCCTGG CTTCAGATT. .CTGGCCTCT GCTT...T.. ..........
GOAT            AGCAGCCTGG CTTCAGATT. .CTGGCCTCT GCTTGGCT.. ..........
HUMAN           TGCAGCCTGG CTTCAGATA. .CTGGCTTCT GCCTGGCTCC TTG......A
CONSENSUS       -GCAGCCTGG CTTCAGATAT -CTGGCTTCT GCCTGGCTCC TTGGG--TA-

                232        MNF-1              NF-1                 281
RAT             TTCCACCTTG TCCTTGCCCA GGTCCAAGAA AAAGCCCAGA ACCTTGGCAC
MOUSE           GTCTACCTTG TCCTTGCCCA GGTCCAAGAA AAGGCCCAGA ACCTTGGCAC
CATTLE          ..CCACTTTG TGCTTTTCAA TGACCAAGAA .AAGCCCAGG CACTTGGAAT
GOAT            ..CCACTTTG TGCTTTTCAA TGACCAAGAA .AATCCCAGG CCCTTGGAAT
HUMAN           TCCCACCCTG CCCTTGTCAG TGACCAAGAA GAAGCCCAGC ACCTTGGCAC
CONSENSUS       TTCCACCTTG TCCTTGTCAA TGACCAAGAA AAAGCCCAG- ACCTTGGCAC
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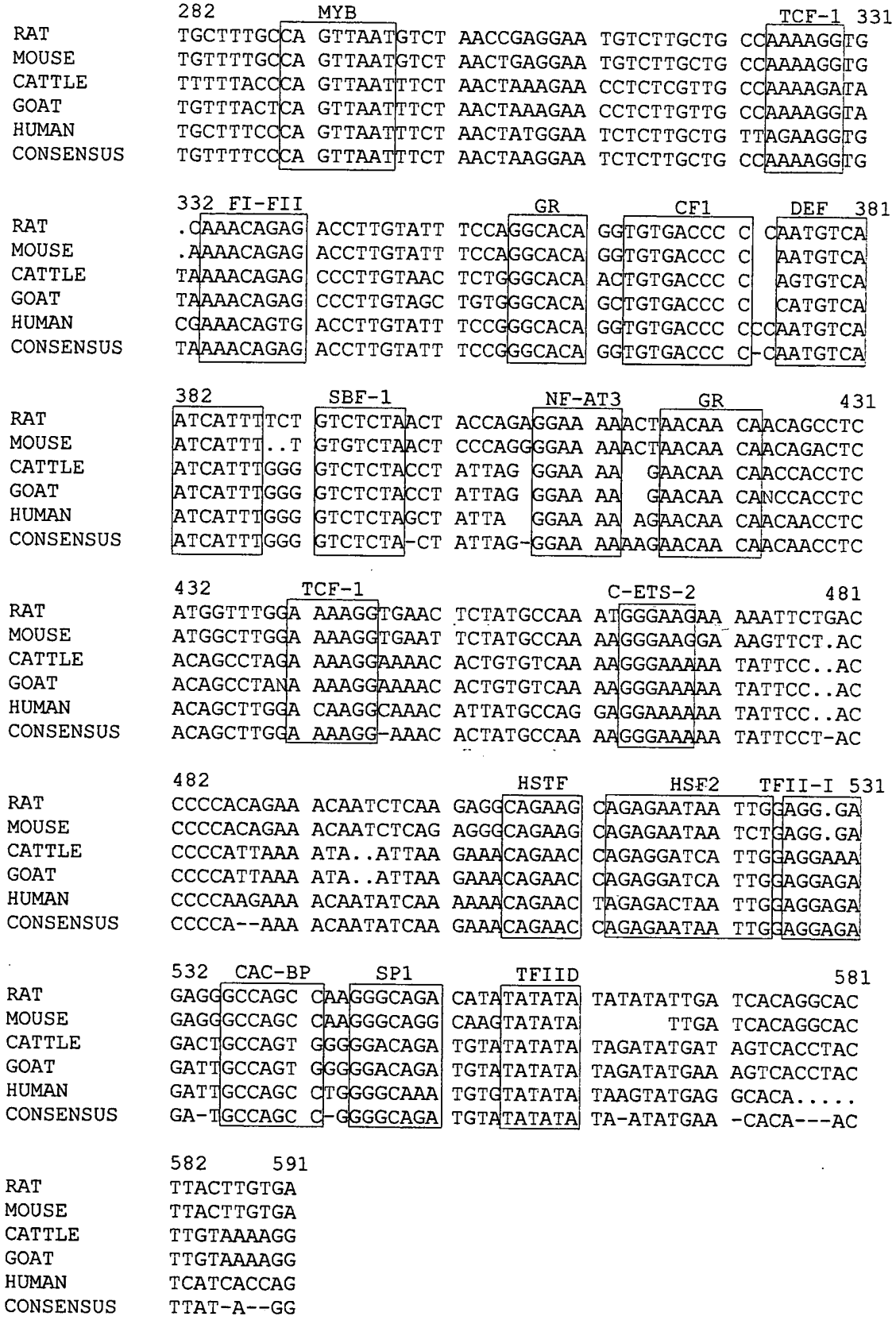
282 MYB TCF-1 331
RAT TGCTTTGCCA GTTAATGTCT AACCGAGGAA TGTCTTGCTG CCAAAAGGTG
MOUSE TGTTTTGCCA GTTAATGTCT AACTGAGGAA TGTCTTGCTG CCAAAAGGTG
CATTLE TTTTTACCCA GTTAATTTCT AACTAAAGAA CCTCTCGTTG CCAAAAGATA
GOAT TGTTTACTCA GTTAATTTCT AACTAAAGAA CCTCTTGTTG CCAAAAGGTA
HUMAN TGCTTTCCCA GTTAATTTCT AACTATGGAA TCTCTTGCTG TTAGAAGGTG
CONSENSUS TGTTTTCCCA GTTAATTTCT AACTAAGGAA TCTCTTGCTG CCAAAAGGTG
332 FI-FII GR CF1 DEF 381
RAT .CAAACAGAG ACCTTGTATT TCCAGGCACA GGTGTGACCC C CAATGTCA
MOUSE .AAAACAGAG ACCTTGTATT TCCAGGCACA GGTGTGACCC C AATGTCA
CATTLE TAAAACAGAG CCCTTGTAAC TCTGGGCACA ACTGTGACCC C AGTGTCA
GOAT TAAAACAGAG CCCTTGTAGC TGTGGGCACA GCTGTGACCC C CATGTCA
HUMAN CGAAACAGTG ACCTTGTATT TCCGGGCACA GGTGTGACCC CCCAATGTCA
CONSENSUS TAAAACAGAG ACCTTGTATT TCCGGGCACA GGTGTGACCC C-CAATGTCA
382 SBF-1 NF-AT3 GR 431
RAT ATCATTTTCT GTCTCTAACT ACCAGAGGAA AAACTAACAA CAACAGCCTC
MOUSE ATCATTT..T GTGTCTAACT CCCAGGGGAA AAACTAACAA CAACAGACTC
CATTLE ATCATTTGGG GTCTCTACCT ATTAG GGAA AA GAACAA CAACCACCTC
GOAT ATCATTTGGG GTCTCTACCT ATTAG GGAA AA GAACAA CANCCACCTC
HUMAN ATCATTTGGG GTCTCTAGCT ATTA GGAA AA AGAACAA CAACAACCTC
CONSENSUS ATCATTTGGG GTCTCTA-CT ATTAG-GGAA AAAAGAACAA CAACAACCTC
432 TCF-1 C-ETS-2 481
RAT ATGGTTTGGA AAAGGTGAAC TCTATGCCAA ATGGGAAGAA AAATTCTGAC
MOUSE ATGGCTTGGA AAAGGTGAAT TCTATGCCAA AAGGGAAGGA AAGTTCT.AC
CATTLE ACAGCCTAGA AAAGGAAAAC ACTGTGTCAA AAGGGAAAAA TATTCC..AC
GOAT ACAGCCTANA AAAGGAAAAC ACTGTGTCAA AAGGGAAAAA TATTCC..AC
HUMAN ACAGCTTGGA CAAGGCAAAC ATTATGCCAG GAGGAAAAAA TATTCC..AC
CONSENSUS ACAGCTTGGA AAAGG-AAAC ACTATGCCAA AAGGGAAAAA TATTCCT-AC
482 HSTF HSF2 TFII-I 531
RAT CCCCACAGAA ACAATCTCAA GAGGCAGAAG CAGAGAATAA TTGGAGG.GA
MOUSE CCCCACAGAA ACAATCTCAG AGGGCAGAAG CAGAGAATAA TCTGAGG.GA
CATTLE CCCCATTAAA ATA..ATTAA GAAACAGAAC CAGAGGATCA TTGGAGGAAA
GOAT CCCCATTAAA ATA..ATTAA GAAACAGAAC CAGAGGATCA TTGGAGGAGA
HUMAN CCCCAAGAAA ACAATATCAA AAAACAGAAC TAGAGACTAA TTGGAGGAGA
CONSENSUS CCCCA--AAA ACAATATCAA GAAACAGAAC CAGAGAATAA TTGGAGGAGA
532 CAC-BP SP1 TFIID 581
RAT GAGGGCCAGC CAAGGGCAGA CATATATATA TATATATTGA TCACAGGCAC
MOUSE GAGGGCCAGC CAAGGGCAGG CAAGTATATA TTGA TCACAGGCAC
CATTLE GACTGCCAGT GGGGGACAGA TGTATATATA TAGATATGAT AGTCACCTAC
GOAT GATTGCCAGT GGGGGACAGA TGTATATATA TAGATATGAA AGTCACCTAC
HUMAN GATTGCCAGC CTGGGGCAAA TGTGTATATA TAAGTATGAG GCACA.....
CONSENSUS GA-TGCCAGC C-GGGGCAGA TGTATATATA TA-ATATGAA -CACA---AC
582 591
RAT TTACTTGTGA
MOUSE TTACTTGTGA
CATTLE TTGTAAAAGG
GOAT TTGTAAAAGG
HUMAN TCATCACCAG
CONSENSUS TTAT-A--GG

Sequence Identity of Uromodulin Promoters of Different Mammals

	Mouse	Rat	Goat	Cattle	Human
Mouse	N/C	90%	74%	73%	77%
Rat	90%	N/C	73%	74%	73%
Goat	74%	73%	N/C	**94%**	76%
Cattle	73%	74%	**94%**	N/C	78%
Human	77%	73%	76%	78%	N/C

Identity between sequence pairs are indicated as percentage identity. Note that promoters of two farm animals, goat and cattle, share 94% identity.
N/C: Sequences from the same species are 100% identical and are thus not compared.

Mouse versus rat

BestFit Results

Refine

BESTFIT of: m.THP.promoter.log check: 5833 from: 1 to: 2092

REFORMAT of: m.THP.promoter.log.29746 check: 5833 from: 1 to: 2092
February 18, 2001 21:38
(No documentation)

to: rat589 check: 5372 from: 1 to: 581

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000
Quality:	4177	Length:	585
Ratio:	7.315	Gaps:	8
Percent Similarity:	90.459	Percent Identity:	90.459

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

m.THP.promoter.log x rat589 September 22, 2004 10:34 ..

```
1522 AGTCTTGTCTGACAGAGGTCCAGTTCAGGAGTGTCCAGATGGTCTGATAA 1571
     ||||||||||||||||||||||||| |||  |||||||||||||   ||
   2 agtcttgtctgacagaggtccagttgagggatgtccagatggtcttgcaa 51

1572 CCTGATGCCATTCTCAGAGA....CTCTTTCCTGTCTGGAATCTAGTGAG 1617
     || |||  | |||||||||    ||||||||||||||| ||||||| |
  52 cc.gataactttctcagagactctctctttcctgtctggactctagtggg 100

1618 GAGGACTTATCTGGTGAAGCTGTCCTTTAGAACAGGAGTGTGTTCCAGTC 1667
     ||||||| |||||||||||||| ||| ||| |||| |||||||||||  |
 101 gaggactaatctggtgaagctgttcttcagatcaggtgtgtgttccaggc 150

1668 TTCAAAGCAAACATTCCTTTTATCCTAACACAGTCTGACTTCAGATATAC 1717
     ||| |||||||  || || |||||||||| ||| ||| ||||||||||
 151 ttcgaagcaaatgtttctgttatcctaacccaggctggcttcagatat.. 198

1718 TGTCTTTTTCCTGGCTCCTTGGGCTTAGGTCTACCTTGTCCTTGCCCAGG 1767
     ||||||||| |  || |||| ||  ||  || ||||||||||||||||||
 199 tgtctttttttcctgccccttttgg..tatttccaccttgtccttgcccagg 246

1768 TCCAAGAAAAGGCCCAGAACCTTGGCACTGTTTTGCCAGTTAATGTCTAA 1817
     |||||||||| |||||||||||||||||| ||||||||||||||||||
 247 tccaagaaaaagcccagaaccttggcactgctttgccagttaatgtctaa 296

1818 CTGAGGAATGTCTTGCTGCCAAAAGGTGAAAACAGAGACCTTGTATTTCC 1867
     | |||||||||||||||||||||||| ||||||||||||||||||||
```

```
 297 ccgaggaatgtcttgctgccaaaaggtgcaaacagagaccttgtatttcc 346
                .         .         .         .         .
1868 AGGCACAGGTGTGA.CCCCAATGTCAATCATTT..TGTGTCTAACTCCCA 1914
     ||||||||||||||| |||||||||||||||||  ||| ||||||| |||
 347 aggcacaggtgtgacccccaatgtcaatcattttctgtctctaactacca 396
                .         .         .         .         .
1915 GGGGAAAAACTAACAACAACAGACTCATGGCTTGGAAAAGGTGAATTCTA 1964
     | |||||||||||||||||||| ||||||| |||||||||||||| ||||
 397 gaggaaaaactaacaacaacagcctcatggtttggaaaaggtgaactcta 446
                .         .         .         .         .
1965 TGCCAAAAGGGAAGGAAAGTTCT.ACCCCCACAGAAACAATCTCAGAGGG 2013
     ||||||| |||||| ||| |||| ||||||||||||||||||||   ||
 447 tgccaaatgggaagaaaaattctgacccccacagaaacaatctcaagagg 496
                .         .         .         .         .
2014 CAGAAGCAGAGAATAATCTGAGGGAGAGGGCCAGCCAAGGGCAGGC.... 2059
     |||||||||||||||||  |||||||||||||||||||||||||| |
 497 cagaagcagagaataattggagggagagggccagccaagggcagacatat 546
                .         .         .
2060 ..AAGTATATATTGATCACAGGCACTTACTTGTGA 2092
       |  ||||||||||||||||||||||||||||||
 547 atatatatatattgatcacaggcacttacttgtga 581
```

Input Sequence: m.THP.promoter.log

```
!!NA_SEQUENCE 1.0
 REFORMAT of: m.THP.promoter.log.29746  check: 5833  from: 1
to: 2092  February 18, 2001 21:38

 (No documentation)

m.THP.promoter.log  Length: 2092  February 18, 2001 21:38
Type: N  Check: 5833  ..

       1  AATTTAAGAC TGGTTGATTT CTTGAATTTC AGTGGGCTTG
```

View Sequence

Input Sequence: rat589

```
!!NA_SEQUENCE 1.0

rat589  Length: 581  September 18, 2001 10:45  Type: N
Check: 5372  ..

       1  tagtcttgtc tgacagaggt ccagttgagg gatgtccaga
tggtcttgca

      51  accgataact ttctcagaga ctctctcttt cctgtctgga
ctctagtggg
```

View Sequence

Mouse versus goat

BestFit Results

Refine

BESTFIT of: m.THP.promoter.log check: 5833 from: 1 to: 2092

REFORMAT of: m.THP.promoter.log.29746 check: 5833 from: 1 to: 2092
February 18, 2001 21:38
(No documentation)

to: g.THP.promoter18.log check: 4545 from: 1 to: 1630

REFORMAT of: g.THP.promoter18.log.22665 check: 4545 from: 1 to: 1630
February 18, 2001 21:37
(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000
Quality:	1374	Length:	422
Ratio:	3.444	Gaps:	11
Percent Similarity:	74.300	Percent Identity:	73.791

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

m.THP.promoter.log x g.THP.promoter18.log September 22, 2004 10:32 ..

```
1677 AACATTCCTTTTATCCTAACACAGTCTGACTTCAGATATACTGTCTTTTT 1726
     | ||  |||||| ||||    ||| ||| |||||||             |
1121 AGCACCCCTTTTCTCCTGGAGCAGCCTGGCTTCAGA.............T 1157

1727 CCTGGCTCCTTGGGCTTAGGTCTACCTTGTCCTTGCCCAGGTCCAAGAAA 1776
      |||||  ||   |||| | || || |||| |||  | | | ||||||||
1158 TCTGGCCTCT...GCTTGGCTCCACTTTGTGCTTTTCAATGACCAAGAAA 1204

1777 AGGCCCAGAACCTTGGCACTGTTTTGCCAGTTAATGTCTAACTGAGGAAT 1826
     |  |||||  |||||| | |||||   |||||||| ||||||| | |||
1205 A.TCCCAGGCCCTTGGAATTGTTTACTCAGTTAATTTCTAACTAAAGAAC 1253

1827 GTCTTGCTGCCAAAAGGT.GAAAACAGAGACCTTGTATTTCCAGGCACAG 1875
      ||||| ||||||||||  |||||||| ||||||| |   |||||||
1254 CTCTTGTTGCCAAAAGGTATAAAACAGAGCCCTTGTAGCTGTGGGCACAG 1303

1876 GTGTGACCCCAATGTCAATCATTT..TGTGTCTAACTCCCAGGGGAAAAA 1923
      ||||||||| ||||||||||||   || |||| ||   ||||   |||
1304 CTGTGACCCCCATGTCAATCATTTGGGGTCTCTACCTATTAGGG...AAA 1350

1924 CTAACAACAACAGACTCATGGCTTGGAAAAGGTGAATTCTATGCCAAAAG 1973
       |||||||:|   ||||  || | :||||||  ||  || || ||||||
1351 AGAACAACANCCACCTCACAGCCTANAAAAGGAAAACACTGTGTCAAAAG 1400
```

```
1974 GGAAGGAAAGTTCTACCCCCACAGAAACAATCTCAGAGGGCAGAAGCAGA 2023
     ||||  ||  ||| |||||||   ||| ||| | |||   ||||| ||||
1401 GGAA.AAATATTCCACCCCCATTAAAATAAT.TAAGA.AACAGAACCAGA 1447
          .         .         .         .         .
2024 GAATAATCTGAGG.GAGAGGGCCAGCCAAGGGCAG..GCAAGTATATATT 2070
     | || ||  |||| ||||  |||||    || |||  | |  ||||||
1448 GGATCATTGGAGGAGAGATTGCCAGTGGGGGACAGATGTATATATATAGA 1497
          .         .
2071 GATCACAGGCACTTACTTGTGA 2092
      || | || ||| ||||||| |
1498 TATGAAAGTCACCTACTTGTAA 1519
```

Input Sequence: m.THP.promoter.log

```
!!NA_SEQUENCE 1.0
 REFORMAT of: m.THP.promoter.log.29746  check: 5833  from: 1
to: 2092  February 18, 2001 21:38

 (No documentation)

m.THP.promoter.log  Length: 2092  February 18, 2001 21:38
Type: N  Check: 5833  ..

       1  AATTTAAGAC TGGTTGATTT CTTGAATTTC AGTGGGCTTG
```

View Sequence

Input Sequence: g.THP.promoter18.log

```
!!NA_SEQUENCE 1.0
 REFORMAT of: g.THP.promoter18.log.22665  check: 4545  from:
1  to: 1630  February 18, 2001 21:37

 (No documentation)

g.THP.promoter18.log  Length: 1630  February 18, 2001 21:37
Type: N  Check: 4545  ..

       1  ACTATAGGGC ACGCGTGGTC GACGGCCCGG GCTGGTAAAG
```

View Sequence

Mouse versus cattle

BestFit Results

Refine

BESTFIT of: m.THP.promoter.log check: 5833 from: 1 to: 2092

REFORMAT of: m.THP.promoter.log.29746 check: 5833 from: 1 to: 2092
February 18, 2001 21:38
(No documentation)

to: cattle.THP.promoter.txt check: 7177 from: 1 to: 626

REFORMAT of: cattle.THP.promoter.txt.27851 check: 7177 from: 1 to: 626
February 18, 2001 21:37
(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000
Quality:	1284	Length:	439
Ratio:	3.109	Gaps:	12
Percent Similarity:	72.973	Percent Identity:	72.973

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

m.THP.promoter.log x cattle.THP.promoter.txt September 22, 2004 10:26

```
1660 TTCCAGTCTTCAAAGCAAACATTCCTTTTATCCTAACACAGTCTGACTTC 1709
     | |||||    | ||  | ||  |||||| ||||    ||| ||| ||||
 168 tcccagtggggacagtgagcacccctttctcctggagcagcctggcttc 217

1710 AGATATACTGTCTTTTTCCTGGCTCCTTGGGCTTAGGTCTACCTTGTCCT 1759
     |||             | |||||  ||   ||||   || || |||| ||
 218 aga.............ttctggcctct...gctt...tccactttgtgct 248

1760 TGCCCAGGTCCAAGAAAAGGCCCAGAACCTTGGCACTGTTTTGCCAGTTA 1809
     |  | | | |||||||||| ||||||   |||||  | | |||  |||||||
 249 tttcaatgaccaagaaaa.gcccaggcacttggaatttttacccagtta 297

1810 ATGTCTAACTGAGGAATGTCTTGCTGCCAAAAGGT.GAAAACAGAGACCT 1858
     || ||||||| | |||  ||| | ||||||||| |  |||||||||  |||
 298 atttctaactaaagaacctctcgttgccaaaagatataaaacagagccct 347

1859 TGTATTTCCAGGCACAGGTGTGACCCCAATGTCAATCATTT..TGTGTCT 1906
     ||||  ||  ||||||  |||||||||| ||||||||||||   || |||
 348 tgtaactctgggcacaactgtgaccccagtgtcaatcatttggggtctct 397

1907 AACTCCCAGGGGAAAAACTAACAACAACAGACTCATGGCTTGGAAAAGGT 1956
     | ||   ||||   |||  |||||||||   ||||  || | |||||||
 398 acctattaggg...aaaagaacaacaaccacctcacagcctagaaaagga 444
```

```
1957 GAATTCTATGCCAAAAGGGAAGGAAAGTTCTACCCCCACAGAAACAATCT 2006
      ||  || || ||||||||||  ||  ||| |||||||   ||| ||| |
 445 aaacactgtgtcaaaagggaa.aaatattccacccccattaaaataat.t 492

2007 CAGAGGGCAGAAGCAGAGAATAATCTGAGG.GAGAGGGCCAGCCAAGGGC 2055
      |||   ||||| ||||| || ||  ||||  |||  |||||    || |
 493 aaga.aacagaaccagaggatcattggaggaaagactgccagtgggggac 541

2056 AG..GCAAGTATATATTGATCACAGGCACTTACTTGTGA 2092
      ||  | |  ||||||   || | || ||| ||||||| |
 542 agatgtatatatatagatatgatagtcacctacttgtaa 580
```

Input Sequence: m.THP.promoter.log

```
!!NA_SEQUENCE 1.0
 REFORMAT of: m.THP.promoter.log.29746  check: 5833  from: 1
to: 2092  February 18, 2001 21:38

 (No documentation)

m.THP.promoter.log  Length: 2092  February 18, 2001 21:38
Type: N  Check: 5833  ..

       1  AATTTAAGAC TGGTTGATTT CTTGAATTTC AGTGGGCTTG
```

View Sequence

Input Sequence: cattle.THP.promoter.txt

```
!!NA_SEQUENCE 1.0
 REFORMAT of: cattle.THP.promoter.txt.27851  check: 7177
from: 1  to: 626  February 18, 2001 21:37

 (No documentation)

cattle.THP.promoter.txt  Length: 626  February 18, 2001
21:37  Type: N  Check: 7177  ..

       1  aatttcttga ttcacagagc atctggtcca atgatgtctg
```

View Sequence

Mouse versus human

BestFit Results

Refine

BESTFIT of: m.THP.promoter.log check: 5833 from: 1 to: 2092

REFORMAT of: m.THP.promoter.log.29746 check: 5833 from: 1 to: 2092
February 18, 2001 21:38
(No documentation)

to: human.THP.promoter.txt check: 7451 from: 1 to: 620

REFORMAT of: human.THP.promoter.txt.30475 check: 7451 from: 1 to: 620
February 18, 2001 21:36
(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000
Quality:	1790	Length:	407
Ratio:	4.509	Gaps:	7
Percent Similarity:	76.590	Percent Identity:	76.590

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

m.THP.promoter.log x human.THP.promoter.txt September 22, 2004 10:33 ..

```
                .         .         .         .         .
1682 TCCTTTTATCCTAACACAGTCTGACTTCAGATATACTGTCTTTTTCCTGG 1731
     ||||||| ||||  |  ||| ||| ||||||  |||||| ||| | |||||
 173 tccttttgtccttatgcagcctggcttcag..atactggcttctgcctgg 220
                .         .         .         .         .
1732 CTCCTTGGGCTTAGGTCTACCTTGTCCTTGCCCAGGTCCAAGAAAAGGCC 1781
     |||||||  |       | ||| || ||||| |   | ||||||| | |||
 221 ctccttgatc......ccaccctgcccttgtcagtgaccaagaagaagcc 264
                .         .         .         .         .
1782 CAGAACCTTGGCACTGTTTTGCCAGTTAATGTCTAACTGAGGAATGTCTT 1831
     ||| |||||||||||| ||| ||||||||| |||||||  ||||| ||||
 265 cagcaccttggcactgctttcccagttaatttctaactatggaatctctt 314
                .         .         .         .         .
1832 GCTGCCAAAAGGTG.AAAACAGAGACCTTGTATTTCCAGGCACAGGTGTG 1880
     ||||  | ||||||  |||||| |||||||||||||| ||||||||||||
 315 gctgttagaaggtgcgaaacagtgaccttgtatttccgggcacaggtgtg 364
                .         .         .         .         .
1881 A..CCCCAATGTCAATCATTTTGTGTCTAACTCCCAGGGGAAAAACTAAC 1928
     |  |||||||||||||||||| | ||||    |     |||||||  |||
 365 acccccaatgtcaatcatttggggtctctagctattaggaaaaa.gaac 413
                .         .         .         .         .
1929 AACAACAGACTCATGGCTTGGAAAAGGTGAATTCTATGCCAAAAGGGAAG 1978
     |||||||  ||||  ||||||| ||||  ||   |||||||  | ||||
 414 aacaacaacctcacagcttggacaaggcaaacattatgccagga.ggaaa 462
```

```
                .          .          .          .          .
1979 GAAAGTTCTACCCCCACAGAAACAATCTCAGAGGGCAGAAGCAGAGAATA 2028
     ||  ||| |||||||   |||||||  ||| |   |||||  |||||  ||
 463 aaatattccacccccaagaaaacaatatcaaaaaacagaactagagacta 512
                .          .          .          .          .
2029 ATCTGAGG.GAGAGGGCCAGCCAAGGGCAGGCAAGTATATATTGATCACA 2077
     ||  |||| ||||  |||||||  |||||     ||||||||   |   |
 513 attggaggagagattgccagcctggggcaaatgtgtatatataagtatga 562

2078 GGCACTT 2084
     ||||| |
 563 ggcacat 569
```

Input Sequence: m.THP.promoter.log

```
!!NA_SEQUENCE 1.0
 REFORMAT of: m.THP.promoter.log.29746  check: 5833  from: 1
to: 2092  February 18, 2001 21:38

 (No documentation)

m.THP.promoter.log  Length: 2092  February 18, 2001 21:38
Type: N  Check: 5833  ..

       1  AATTTAAGAC TGGTTGATTT CTTGAATTTC AGTGGGCTTG
```

View Sequence

Input Sequence: human.THP.promoter.txt

```
!!NA_SEQUENCE 1.0
 REFORMAT of: human.THP.promoter.txt.30475  check: 7451
from: 1  to: 620  February 18, 2001 21:36

 (No documentation)

human.THP.promoter.txt  Length: 620  February 18, 2001 21:36
Type: N  Check: 7451  ..

       1  cagagtgggt caggtccagt gatgtctgaa ctaccttctg
```

View Sequence

BestFit Results

Rat versus goat

Refine

BESTFIT of: Rat.THP.promoter.txt check: 1164 from: 1 to: 625

REFORMAT of: Rat.THP.promoter.txt.27903 check: 1164 from: 1 to: 625
February 18, 2001 21:37
(No documentation)

to: g.THP.promoter18.log check: 4545 from: 1 to: 1630

REFORMAT of: g.THP.promoter18.log.22665 check: 4545 from: 1 to: 1630
February 18, 2001 21:37
(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000
Quality:	1640	Length:	462
Ratio:	3.744	Gaps:	9
Percent Similarity:	73.733	Percent Identity:	73.272

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

Rat.THP.promoter.txt x g.THP.promoter18.log September 22, 2004 11:11

```
  168 ctgttatcctaacccaggctggcttcagatattgtctttttcctgcccc 217
      || || ||||    ||| ||||||||||           | ||| ||
 1128 CTTTTCTCCTGGAGCAGCCTGGCTTCAGA.....TTCTGGCCT 1165

  218 tttggtatttccaccttgtccttgcccaggtccaagaaaaagcccagaac 267
       |   |  ||||| |||| |||  | | | ||||| |||| |||||  |
 1166 CTGCTTGGCTCCACTTTGTGCTTTTCAATGACCAAG.AAAATCCCAGGCC 1214

  268 cttggcactgctttgccagttaatgtctaaccgaggaatgtcttgctgcc 317
      ||||| | || ||   |||||||| ||||||  | |||  ||||| ||||
 1215 CTTGGAATTGTTTACTCAGTTAATTTCTAACTAAAGAACCTCTTGTTGCC 1264

  318 aaaaggt.gcaaacagagaccttgtatttccaggcacaggtgtgaccccc 366
      |||||||  |||||||| |||||||  |  ||||||| ||||||||||
 1265 AAAAGGTATAAAACAGAGCCCTTGTAGCTGTGGGCACAGCTGTGACCCCC 1314

  367 aatgtcaatcattttctgtctctaactaccagaggaaaaactaacaacaa 416
       |||||||||||||  ||||||| |||  || |   |||  |||||||:
 1315 .ATGTCAATCATTTGGGGTCTCTACCTATTAGGG...AAAAGAACAACAN 1360

  417 cagcctcatggtttggaaaaggtgaactctatgccaaatgggaagaaaaa 466
      |  |||||  |  | :|||||  ||| || || |||| ||||| || |
 1361 CCACCTCACAGCCTANAAAAGGAAAACACTGTGTCAAAAGGGAAAAATAT 1410
```

Rat versus cattle

BestFit Results

Refine

BESTFIT of: Rat.THP.promoter.txt check: 1164 from: 1 to: 625

REFORMAT of: Rat.THP.promoter.txt.27903 check: 1164 from: 1 to: 625
February 18, 2001 21:37
(No documentation)

to: cattle.THP.promoter.txt check: 7177 from: 1 to: 626

REFORMAT of: cattle.THP.promoter.txt.27851 check: 7177 from: 1 to: 626
February 18, 2001 21:37
(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000
Quality:	1598	Length:	461
Ratio:	3.682	Gaps:	10
Percent Similarity:	74.186	Percent Identity:	74.186

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

Rat.THP.promoter.txt x cattle.THP.promoter.txt September 22, 2004 11:13

```
168 ctgttatcctaacccaggctggcttcagatattgtctttttcctgcccc 217
    || || ||||    ||| ||||||||||||  || | |     ||||
192 cttttctcctggagcagcctggcttcagattctggcct.....ctgc... 233

218 tttggtatttccaccttgtccttgcccaggtccaagaaaaagcccagaac 267
          ||||||| |||| |||  | | | ||||| ||||||||||
234 .......tttccactttgtgcttttcaatgaccaag.aaaagcccaggca 275

268 cttggcactgctttgccagttaatgtctaaccgaggaatgtcttgctgcc 317
    ||||| | |  ||  |||||||||| ||||||  | |||  ||| | ||||
276 cttggaatttttacccagttaatttctaactaaagaacctctcgttgcc 325

318 aaaaggt.gcaaacagagaccttgtatttccaggcacaggtgtgaccccc 366
    ||||| |   |||||||| |||||||  ||  ||||||  ||||| ||||
326 aaaagatataaaacagagcccttgtaactctgggcacaactgtga.cccc 374

367 aatgtcaatcattttctgtctctaactaccagaggaaaaactaacaacaa 416
    | ||||||||||||   ||||||| |||  || |   |||  ||||||||
375 agtgtcaatcatttggggtctctacctattaggg...aaaagaacaacaa 421

417 cagcctcatggtttggaaaaggtgaactctatgccaaatgggaagaaaaa 466
    |  |||||  |  | |||||||  ||| || || |||| ||||| || |
422 ccacctcacagcctagaaaaggaaaacactgtgtcaaaagggaaaaatat 471
```

```
                .          .          .          .          .
467 ttctgacccccacagaaacaatctcaagaggcagaagcagagaataattg 516
    | |  |||||||   |||  ||  | ||||  ||||| ||||| || ||||
472 tcc..acccccattaaaataa..ttaagaaacagaaccagaggatcattg 517
                .          .          .          .          .
517 gagggagagggccagccaagggcagacatatatatatatatattgatcac 566
    |||   ||  | | ||||  ||  |||| || ||||||||||   || |
518 gag...gaaagactgccagtgggggacagatgtatatatatagatatgat 564
                .          .          .          .          .
567 aggcacttacttgtgaatggaccagtcct...gtcctgggttcaggtaag 613
    || ||| ||||||| || |||  | | ||    | |||| ||||||||||
565 agtcacctacttgtaaaaggattaattctacctttctggtttcaggtaag 614
                .
614 actgtctggag 624
     || |||| ||
615 gctatctgcag 625
```

Input Sequence: Rat.THP.promoter.txt

```
!!NA_SEQUENCE 1.0
 REFORMAT of: Rat.THP.promoter.txt.27903  check: 1164  from:
1  to: 625  February 18, 2001 21:37

 (No documentation)

Rat.THP.promoter.txt  Length: 625  February 18, 2001 21:37
Type: N  Check: 1164  ..

       1  ctagtcttgt ctgacagagg tccagttgag ggatgtccag
```

View Sequence

Input Sequence: cattle.THP.promoter.txt

```
!!NA_SEQUENCE 1.0
 REFORMAT of: cattle.THP.promoter.txt.27851  check: 7177
from: 1  to: 626  February 18, 2001 21:37

 (No documentation)

cattle.THP.promoter.txt  Length: 626  February 18, 2001
21:37  Type: N  Check: 7177  ..

       1  aatttcttga ttcacagagc atctggtcca atgatgtctg
```

View Sequence

Rat versus human

BestFit Results

Refine

BESTFIT of: Rat.THP.promoter.txt check: 1164 from: 1 to: 625

REFORMAT of: Rat.THP.promoter.txt.27903 check: 1164 from: 1 to: 625
February 18, 2001 21:37
(No documentation)

to: human.THP.promoter.txt check: 7451 from: 1 to: 620

REFORMAT of: human.THP.promoter.txt.30475 check: 7451 from: 1 to: 620
February 18, 2001 21:36
(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000
Quality:	2040	Length:	588
Ratio:	3.611	Gaps:	10
Percent Similarity:	73.297	Percent Identity:	73.297

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

Rat.THP.promoter.txt x human.THP.promoter.txt September 22, 2004 11:14

```
     60 ctttctcagagactctctctttcctgtctggactctagtggggaggacta 109
     56 cattctcagctcctctcttgcttgtgtctggattct.......aaggctg 98

    110 atctggtgaagctg..................ttcttcagatcaggtgt 140
     99 atctcatgagaatgggtgtttcagaagggtgccctctccaagacaggtgc 148

    141 gtgttccaggcttcgaagcaaatgtttctgttatcctaacccaggctggc 190
    149 acctcccatctggggcagtgaat.atccttttgtccttatgcagcctggc 197

    191 ttcagatattgtctttttttcctgccccttggtatttccaccttgtcctt 240
    198 ttcagatactggcttctgcctggctcct....tgatcccaccctgccctt 243

    241 gcccaggtccaagaaaaagcccagaaccttggcactgctttgccagttaa 290
    244 gtcagtgaccaagaagaagcccagcaccttggcactgctttcccagttaa 293

    291 tgtctaaccgaggaatgtcttgctgccaaaaggtgc.aaacagagacctt 339
    294 tttctaactatggaatctcttgctgttagaaggtgcgaaacagtgacctt 343
```

```
                .          .          .          .          .
340 gtatttccaggcacaggtgtga.cccccaatgtcaatcattttctgtctc 388
    |||||||| |||||||||||||| ||||||||||||||||||    |||||
344 gtatttccgggcacaggtgtgacccccccaatgtcaatcatttggggtctc 393
          .          .          .          .          .
389 taactaccagaggaaaaactaacaacaacagcctcatggtttggaaaagg 438
    || |||  |   | ||||  |||||||||| |||||  | |||||  |||| 
394 tagctatta...ggaaaaagaacaacaacaacctcacagcttggacaagg 440
          .          .          .          .          .
439 tgaactctatgccaaatgggaagaaaaattctgacccccacagaaacaat 488
      |||  |||||||   || || || | | |  |||||||   ||||||| 
441 caaacattatgccaggaggaaaaaatattcc..acccccaagaaaacaat 488
          .          .          .          .          .
489 ctcaagaggcagaagcagagaataattggagg.gagagggccagccaagg 537
     |||| |  |||||  ||||| |||||||||| ||||  |||||||  || 
489 atcaaaaaacagaactagagactaattggaggagagattgccagcctggg 538
          .          .          .          .          .
538 gcagacatatatatatatattgatcacaggcacttacttgtgaatgga 587
    ||| |  | ||||||||  |||       |||||| |  |    |    | 
539 gcaaatgtgtatatataagtat......gaggcacatcatcaccagacta 582
          .          .          .
588 ccagtcctgtcctgggttcaggtaagactgtctggagc 625
     |  | |  | |||| |||||||||| || |||| ||| 
583 actctacctttctggcttcaggtaaggctatctgtagc 620
```

Input Sequence: Rat.THP.promoter.txt

```
!!NA_SEQUENCE 1.0
 REFORMAT of: Rat.THP.promoter.txt.27903  check: 1164  from:
1  to: 625  February 18, 2001 21:37

 (No documentation)

Rat.THP.promoter.txt  Length: 625  February 18, 2001 21:37
Type: N  Check: 1164  ..

      1  ctagtcttgt ctgacagagg tccagttgag ggatgtccag
```

View Sequence

Input Sequence: human.THP.promoter.txt

```
!!NA_SEQUENCE 1.0
 REFORMAT of: human.THP.promoter.txt.30475  check: 7451
from: 1  to: 620  February 18, 2001 21:36

 (No documentation)

human.THP.promoter.txt  Length: 620  February 18, 2001 21:36
Type: N  Check: 7451  ..

       1  cagagtgggt caggtccagt gatgtctgaa ctaccttctg
```

View Sequence

goat versus cattle

BestFit Results

Refine

BESTFIT of: g.THP.promoter18.log check: 4545 from: 1 to: 1630

REFORMAT of: g.THP.promoter18.log.22665 check: 4545 from: 1 to: 1630
February 18, 2001 21:37
(No documentation)

to: cattle.THP.promoter.txt check: 7177 from: 1 to: 626

REFORMAT of: cattle.THP.promoter.txt.27851 check: 7177 from: 1 to: 626
February 18, 2001 21:37
(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000
Quality:	5545	Length:	628
Ratio:	8.872	Gaps:	1
Percent Similarity:	94.560	Percent Identity:	94.240

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

g.THP.promoter18.log x cattle.THP.promoter.txt September 22, 2004 10:36

```
                .         .         .         .         .
  937 AATTTCTTGATTCACAGAGCATCTGGTCCAATGATGTCTGAATTATCTGC 986
      |||||||||||||||||||||||||||||||||||||||||||||||||  || |
    1 aatttcttgattcacagagcatctggtccaatgatgtctgaattgccttc 50
                .         .         .         .         .
  987 TGTCTCTGACCTTCAGCCATTCTCAGCTCCTTTCCTGATCACATTGGGAC 1036
      ||||||||||||||| |||||||||||||||||||| |||||| |||||
   51 tgtctctgaccttcaggcattctcagctcctttcctgctcacatcgggac 100
                .         .         .         .         .
 1037 CCCAGGGGAGCTGGCTGAATCTGTGAGGATGGCATTTGCTTTGGAATTAA 1086
      ||||||| |||||| |||| |  ||||||||| | |||||||||| | |
  101 cccagggaagctggttgaacccatgaggatggaacttgctttggaactga 150
                .         .         .         .         .
 1087 GTGGCCACAAGTACACATCCTGGTGGGGACGATGAGCACCCCTTTTCTCC 1136
      ||||||||||||| ||||||  ||||||||  ||||||||||||||||||
  151 gtggccacaagtatacatcccagtggggacagtgagcacccctttctcc 200
                .         .         .         .         .
 1137 TGGAGCAGCCTGGCTTCAGATTCTGGCCTCTGCTTGGCTCCACTTTGTGC 1186
      |||||||||||||||||||||||||||||||||||   ||||||||||||
  201 tggagcagcctggcttcagattctggcctctgctt...tccactttgtgc 247
                .         .         .         .         .
 1187 TTTTCAATGACCAAGAAAATCCCAGGCCCTTGGAATTGTTTACTCAGTTA 1236
      |||||||||||||||||| ||||||| ||||||||| ||||| ||||||
  248 ttttcaatgaccaagaaaagcccaggcacttggaatttttacccagtta 297
```

```
1237 ATTTCTAACTAAAGAACCTCTTGTTGCCAAAAGGTATAAAACAGAGCCCT 1286
     ||||||||||||||||||||| |||||||||| ||||||||||||||||
 298 atttctaactaaagaacctctcgttgccaaaagatataaaacagagccct 347

1287 TGTAGCTGTGGGCACAGCTGTGACCCCCATGTCAATCATTTGGGGTCTCT 1336
     |||| || |||||||| |||||||||  ||||||||||||||||||||
 348 tgtaactctgggcacaactgtgaccccagtgtcaatcatttggggtctct 397

1337 ACCTATTAGGGAAAAGAACAACANCCACCTCACAGCCTANAAAAGGAAAA 1386
     ||||||||||||||||||||||:|||||||||||||:||||||||||
 398 acctattagggaaaagaacaacaaccacctcacagcctagaaaaggaaaa 447

1387 CACTGTGTCAAAAGGGAAAAATATTCCACCCCCATTAAAATAATTAAGAA 1436
     ||||||||||||||||||||||||||||||||||||||||||||||||
 448 cactgtgtcaaaagggaaaaatattccacccccattaaaataattaagaa 497

1437 ACAGAACCAGAGGATCATTGGAGGAGAGATTGCCAGTGGGGGACAGATGT 1486
     |||||||||||||||||||||||| ||| ||||||||||||||||||||
 498 acagaaccagaggatcattggaggaaagactgccagtgggggacagatgt 547

1487 ATATATATAGATATGAAAGTCACCTACTTGTAAAAGGATTAATTCTACCT 1536
     |||||||||||||||| |||||||||||||||||||||||||||||||
 548 atatatatagatatgatagtcacctacttgtaaaaggattaattctacct 597

1537 TTCTGGTTTCAGGTAAGGCTATCTGCAG 1564
     |||||||||||||||||||||||||||
 598 ttctggtttcaggtaaggctatctgcag 625
```

Input Sequence: g.THP.promoter18.log

```
!!NA_SEQUENCE 1.0
 REFORMAT of: g.THP.promoter18.log.22665  check: 4545  from:
1  to: 1630  February 18, 2001 21:37

 (No documentation)

g.THP.promoter18.log  Length: 1630  February 18, 2001 21:37
Type: N  Check: 4545  ..

       1  ACTATAGGGC ACGCGTGGTC GACGGCCCGG GCTGGTAAAG
```

View Sequence

Input Sequence: cattle.THP.promoter.txt

```
!!NA_SEQUENCE 1.0
 REFORMAT of: cattle.THP.promoter.txt.27851  check: 7177
from: 1  to: 626  February 18, 2001 21:37

 (No documentation)

cattle.THP.promoter.txt  Length: 626  February 18, 2001
21:37  Type: N  Check: 7177  ..

       1  aatttcttga ttcacagagc atctggtcca atgatgtctg
```

View Sequence

BestFit Results Goat versus human

Refine

BESTFIT of: g.THP.promoter18.log check: 4545 from: 1 to: 1630

REFORMAT of: g.THP.promoter18.log.22665 check: 4545 from: 1 to: 1630
February 18, 2001 21:37
(No documentation)

to: human.THP.promoter.txt check: 7451 from: 1 to: 620

REFORMAT of: human.THP.promoter.txt.30475 check: 7451 from: 1 to: 620
February 18, 2001 21:36
(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000
Quality:	2836	Length:	633
Ratio:	4.596	Gaps:	8
Percent Similarity:	76.325	Percent Identity:	75.993

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

g.THP.promoter18.log x human.THP.promoter.txt September 22, 2004 11:22

```
                .         .         .         .         .
    949 CACAGAGCATCTGGTCCAATGATGTCTGAATTATCTGCTGTCTCTGACCT 998
        || || |  || |||||| |||||||||||| || || |||  |||||| |
      1 cagagtgggtcaggtccagtgatgtctgaactaccttctggttctgactt 50
                .         .         .         .         .
    999 TCAGCCATTCTCAGCTCCTTTCCTGATCACATTGGGACCCCAGGGGAGCT 1048
        |||||||||||||||||| || || |    |  |||  | |
     51 tcagccattctcagctcctctcttgcttgtgtctggattcta.......a 93
                .         .         .         .         .
   1049 GGCTGAATCTGTGAGGATGGCATTTGCTTTGGAATTAAGTGGCC...ACA 1095
        ||||||     |||| ||||   || |    |  |    |  ||   |||
     94 ggctgatctcatgagaatgggtgtttcagaagggtgccctctccaagaca 143
                .         .         .         .         .
   1096 AGTACACATCCTGGTGGGGACGATGAGCACCCCTTTTCTCCTGGAGCAGC 1145
         || ||| |||     ||| |  |||  |  |||||| ||||   |||||
    144 ggtgcacctcccatctggggcagtgaata.tccttttgtccttatgcagc 192
                .         .         .         .         .
   1146 CTGGCTTCAGATTCTGGCCTCTGCTTGGCT........CCACTTTGTGCT 1187
        ||||||||||| ||||| ||||| |||||         ||||  ||  ||
    193 ctggcttcagatactggcttctgcctggctccttgatcccaccctgccct 242
                .         .         .         .         .
   1188 TTTCAATGACCAAGAA.AATCCCAGGCCCTTGGAATTGTTTACTCAGTTA 1236
        | ||| |||||||||| || |||||  |||||| | || || | ||||||
    243 tgtcagtgaccaagaagaagcccagcaccttggcactgctttcccagtta 292
```

```
1237 ATTTCTAACTAAAGAACCTCTTGTTGCCAAAAGGTATAAAACAGAGCCCT 1286
     ||||||||||  ||| ||||||  ||  | |||||   ||||||  | |||
 293 atttctaactatggaatctcttgctgttagaaggtgcgaaacagtgacct 342

1287 TGTAGCTGTGGGCACAGCTGTGACCCCC..ATGTCAATCATTTGGGGTCT 1334
     ||||  |  |||||||| ||||||||||  ||||||||||||||||||||
 343 tgtatttccgggcacaggtgtgacccccaatgtcaatcatttggggtct 392

1335 CTACCTATTAGGGAAAAGAACAACANCCACCTCACAGCCTANAAAAGGAA 1384
     |||  |||||||| ||||||||||||:| |||||||||| | :| |||| |
 393 ctagctattaggaaaaagaacaacaacaacctcacagcttggacaaggca 442

1385 AACACTGTGTCAAAAGGGAAAAATATTCCACCCCCATTAAAATA..ATTA 1432
     ||||  |  ||  ||  |||  ||||||||||||||||||  ||||  |  ||  |
 443 aacattatgccaggaggaaaaaatattccacccccaagaaaacaatatca 492

1433 AGAAACAGAACCAGAGGATCATTGGAGGAGAGATTGCCAGTGGGGGACAG 1482
     |  |||||||||  ||||  |  ||||||||||||||||||||   |||  ||
 493 aaaaacagaactagagactaattggaggagagattgccagcctggggcaa 542

1483 ATGTATATATATAGATATGAAAGTCACCTACTTGTAAAAGGATTAATTCT 1532
     ||||  ||||||||  |||||    ||     |  | |   ||  |||  |||
 543 atgtgtatatataagtatgaggcaca.....tcatcaccagactaactct 587

1533 ACCTTTCTGGTTTCAGGTAAGGCTATCTGCAGC 1565
     ||||||||||  ||||||||||||||||||  |||
 588 acctttctggcttcaggtaaggctatctgtagc 620
```

Input Sequence: g.THP.promoter18.log

```
!!NA_SEQUENCE 1.0
 REFORMAT of: g.THP.promoter18.log.22665  check: 4545  from:
1  to: 1630  February 18, 2001 21:37

 (No documentation)

g.THP.promoter18.log  Length: 1630  February 18, 2001 21:37
Type: N  Check: 4545  ..

       1  ACTATAGGGC ACGCGTGGTC GACGGCCCGG GCTGGTAAAG
```

View Sequence

Input Sequence: human.THP.promoter.txt

```
!!NA_SEQUENCE 1.0
 REFORMAT of: human.THP.promoter.txt.30475  check: 7451
from: 1  to: 620  February 18, 2001 21:36

 (No documentation)

human.THP.promoter.txt  Length: 620  February 18, 2001 21:36
Type: N  Check: 7451  ..

       1  cagagtgggt caggtccagt gatgtctgaa ctaccttctg
```

View Sequence

BestFit Results

Cattle versus human

Refine

BESTFIT of: cattle.THP.promoter.txt check: 7177 from: 1 to: 626

REFORMAT of: cattle.THP.promoter.txt.27851 check: 7177 from: 1 to: 626
February 18, 2001 21:37
(No documentation)

to: human.THP.promoter.txt check: 7451 from: 1 to: 620

REFORMAT of: human.THP.promoter.txt.30475 check: 7451 from: 1 to: 620
February 18, 2001 21:36
(No documentation)

Symbol comparison table: swgapdna.cmp CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000
Quality:	2816	Length:	637
Ratio:	4.594	Gaps:	10
Percent Similarity:	77.815	Percent Identity:	77.815

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

cattle.THP.promoter.txt x human.THP.promoter.txt September 22, 2004 11:20

```
     13 cacagagcatctggtccaatgatgtctgaattgccttctgtctctgacct 62
        || || |  || |||||| |||||||||||| | |||||||  |||||| |
      1 cagagtgggtcaggtccagtgatgtctgaactaccttctggttctgactt 50

     63 tcaggcattctcagctcctttcctgctcacatcgggaccccagggaagct 112
        |||| |||||||||||||| || ||||    || |||  | |
     51 tcagccattctcagctcctctcttgcttgtgtctggattcta.......a 93

    113 ggttgaacccatgaggatggaacttgctttggaactgagtggcc...... 156
        || ||| | |||||| ||||    || ||  |||  | ||| ||
     94 ggctgatctcatgagaatgg...gtgtttcagaa..gggtgccctctcca 138

    157 ..acaagtatacatcccagtggggacagtgagcacccctttctcctgga 204
          ||| ||  || |||||   ||| ||||||  |  |||||| ||||
    139 agacaggtgcacctcccatctggggcagtgaata.tccttttgtccttat 187

    205 gcagcctggcttcagattctggcctctgc...........tttccacttt 243
        |||||||||||||||| ||||| |||||           | ||||  |
    188 gcagcctggcttcagatactggcttctgcctggctccttgatcccaccct 237

    244 gtgcttttcaatgaccaagaa.aagcccaggcacttggaatttttaccc 292
        |  ||| ||| |||||||||| ||||||||   ||||| | |  || |||
    238 gcccttgtcagtgaccaagaagaagcccagcaccttggcactgctttccc 287
```

```
                .         .         .         .         .
293 agttaatttctaactaaagaacctctcgttgccaaaagatataaaacaga 342
    ||||||||||||||||  ||| |||| | ||  | ||| |   ||||||
288 agttaatttctaactatggaatctcttgctgttagaaggtgcgaaacagt 337
                .         .         .         .         .
343 gcccttgtaactctgggcacaactgtga..ccccagtgtcaatcatttgg 390
    | |||||||  || |||||||  |||||  ||||| ||||||||||||||
338 gaccttgtatttccgggcacaggtgtgacccccccaatgtcaatcatttgg 387
                .         .         .         .         .
391 ggtctctacctattagggaaaagaacaacaaccacctcacagcctagaaa 440
    |||||||| |||||||| |||||||||||||| |||||||||| | || |
388 ggtctctagctattaggaaaaagaacaacaacaacctcacagcttggaca 437
                .         .         .         .         .
441 aggaaaacactgtgtcaaaagggaaaaatattccacccccattaaaata. 489
    ||| ||||| | || ||  ||| ||||||||||||||||||  |||| |
438 aggcaaacattatgccaggaggaaaaaatattccacccccaagaaaacaa 487
                .         .         .         .         .
490 .attaagaaacagaaccagaggatcattggaggaaagactgccagtgggg 538
     || || |||||||||| ||||  | |||||||||| ||| ||||||   ||
488 tatcaaaaaacagaactagagactaattggaggagagattgccagcctgg 537
                .         .         .         .         .
539 gacagatgtatatatatagatatgatagtcacctacttgtaaaaggatta 588
    | || |||| |||||||| |||||    ||     |  | |   || ||
538 ggcaaatgtgtatatataagtatgaggcaca.....tcatcaccagacta 582
                .         .         .
589 attctacctttctggtttcaggtaaggctatctgcag 625
    | |||||||||||| |||||||||||||||||| ||
583 actctacctttctggcttcaggtaaggctatctgtag 619
```

Input Sequence: cattle.THP.promoter.txt

```
!!NA_SEQUENCE 1.0
 REFORMAT of: cattle.THP.promoter.txt.27851  check: 7177
from: 1  to: 626  February 18, 2001 21:37

 (No documentation)

cattle.THP.promoter.txt  Length: 626  February 18, 2001
21:37  Type: N  Check: 7177  ..

       1  aatttcttga ttcacagagc atctggtcca atgatgtctg
```

View Sequence

Input Sequence: human.THP.promoter.txt

```
!!NA_SEQUENCE 1.0
 REFORMAT of: human.THP.promoter.txt.30475  check: 7451
from: 1  to: 620  February 18, 2001 21:36

 (No documentation)

human.THP.promoter.txt  Length: 620  February 18, 2001 21:36
Type: N  Check: 7451  ..

       1  cagagtgggt caggtccagt gatgtctgaa ctaccttctg
```

View Sequence

A Luciana e Luigi
con tanto

Tamm-Horsfall Glycoprotein: Biology and Clinical Relevance

Franca Serafini-Cessi, MD, Nadia Malagolini, PhD, and Daniela Cavallone, PhD

• **Tamm-Horsfall glycoprotein (THP) is the most abundant urinary protein in mammals. Urinary excretion occurs by proteolytic cleavage of the large ectodomain of the glycosyl phosphatidylinositol–anchored counterpart exposed at the luminal cell surface of the thick ascending limb of Henle's loop. We describe the physical-chemical structure of human THP and its biosynthesis and interaction with other proteins and leukocytes. The clinical relevance of THP reported here includes: (1) involvement in the pathogenesis of cast nephropathy, urolithiasis, and tubulointerstitial nephritis; (2) abnormalities in urinary excretion in renal diseases; and (3) the recent finding that familial juvenile hyperuricemic nephropathy and autosomal dominant medullary cystic kidney disease 2 arise from mutations of the THP gene. We critically examine the literature on the physiological role and mechanism(s) that promote urinary excretion of THP. Some lines of research deal with the in vitro immunoregulatory activity of THP, termed uromodulin when isolated from urine of pregnant women. However, an immunoregulatory function in vivo has not yet been established. In the most recent literature, there is renewed interest in the capacity of urinary THP to compete efficiently with urothelial cell receptors, such as uroplakins, in adhering to type 1 fimbriated *Escherichia coli*. This property supports the notion that abundant THP excretion in urine is promoted in the host by selective pressure to obtain an efficient defense against urinary tract infections caused by uropathogenic bacteria. *Am J Kidney Dis* 42: 658-676.**

INDEX WORDS: Tamm-Horsfall protein (THP); uromodulin; urinary proteins; glycosyl phosphatidylinositol (GPI)–anchored proteins; cast nephropathy; myeloma nephropathy; stone disease; interstitial nephritis; *Escherichia coli*; familial juvenile hyperuricemic nephropathy (FJHN); medullary cystic kidney disease 2 (MCKD2).

IN 1950, IGOR TAMM and Frank Horsfall[1] used a salt-precipitation procedure to isolate a potent inhibitor of viral hemoagglutination from urine of healthy individuals. Subsequently, the same investigators[2] undertook the chemical characterization of mucoprotein and the way in which it inhibits hemoagglutination induced by influenza, mumps, and Newcastle disease viruses. In general terms, the 2 investigators sought to obtain evidence of the (then) putative enzymatic activity associated with viruses and identify inhibitors preventing viruses from binding to susceptible cells. They reported that neuraminidase treatment abolished the inhibitory effect in the viral hemoagglutination assay. This observation persuaded Gottschalk[3] and Odin[4] to analyze the carbohydrate moiety of urinary mucoprotein. Both studies established that carbohydrate content accounts for more than 20% to 25% of mucoprotein weight, and sialic acid is abundantly present. Urinary mucoprotein has since become known as Tamm-Horsfall glycoprotein or Tamm-Horsfall protein (THP).

In 1964, Bayer[5] used electron microscopy to confirm the binding of influenza virus to urinary mucoprotein. Urinary THP was visualized as a network of filaments composed of smaller fibrils with a diameter of 4 to 24 nm, but the length could not be detected because of their tendency to form end-to-end aggregates. In the 1970s, Albert Neuberger's laboratory in London showed that THP: (1) migrates in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as a single molecular unit with an apparent molecular weight of 80 to 90 kd; (2) is the most abundant protein in normal urine, excreted at the rate of approximately 50 mg/d; and (3) is present in urine of other mammals.[6-9]

THP had been constantly under the attention of investigators working in the field of glycoproteins or nephrologists and urologists (according to PubMed from January 1967 to January 1985, almost 200 articles concerning Tamm-Horsfall glycoprotein were published) when, in 1985, it was rediscovered by Muchmore and Decker.[10]

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0272-6386/03/4204-0005$30.00/0
doi:10.1053/S0272-6386(03)00829-1

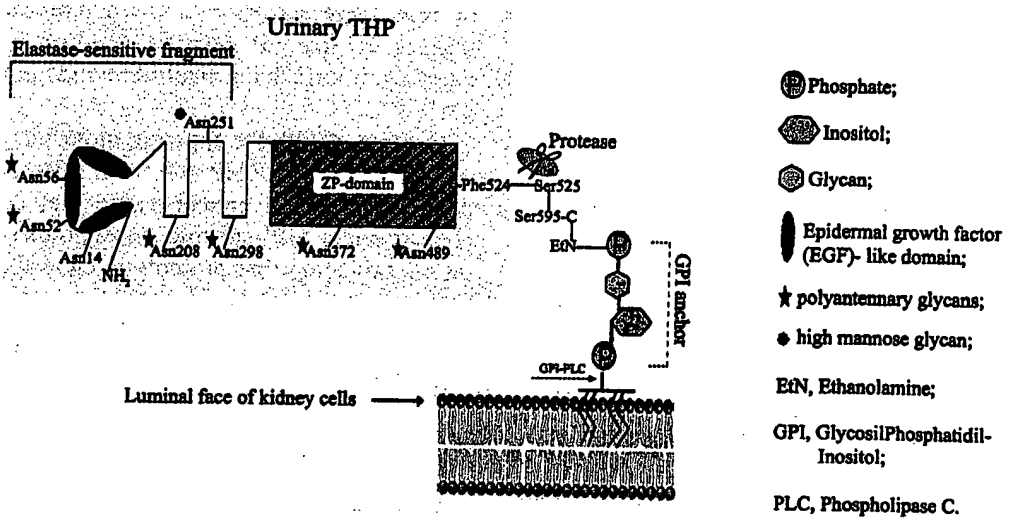

Fig 1. Structural model of urinary THP (yellow area) and its renal GPI-anchored counterpart.

These investigators isolated a glycoprotein from urine of pregnant women by a method that includes lectin-affinity chromatography. They named the protein uromodulin because in some conditions, it showed in vitro immunosuppressive activity. A similar property previously was shown to be present in THP.[11,12] In 1987, the complementary DNA (cDNA) of uromodulin was cloned, and its identity with the cDNA of human THP was shown.[13,14]

PHYSICAL-CHEMICAL STRUCTURE OF URINARY THP AND ITS RENAL GLYCOSYL PHOSPHATIDYLINOSITOL–ANCHORED COUNTERPART

The primary structure of human-THP has been predicted by sequencing the cDNA.[13,14] On the basis of the *N*-terminal sequence (Asp-Thr-Ser-Glu-Ala) found in urinary THP, Pennica et al[13] assigned 616 amino acids to THP, in that the first 24 *N*-terminal amino acids predicted by cDNA represent the signal peptide lacking in the mature protein (we have used amino acid-mapping according to Pennica et al,[13] to indicate both the *N*-glycosylation sites and all cited amino acids throughout the text). The protein includes 48 cysteine residues, 8 potential *N*-glycosylation sites, 3 epidermal growth factor domains, 1 zona pellucida (ZP)-like domain, and, at the C-terminus, 1 stretch of hydrophobic amino acids, similar to that of proteins that acquire a glycosyl phosphatidylinositol (GPI) anchor. This hydrophobic sequence acts as a signal for endoplasmic reticulum (ER)-transpeptidase, which, after cleav-

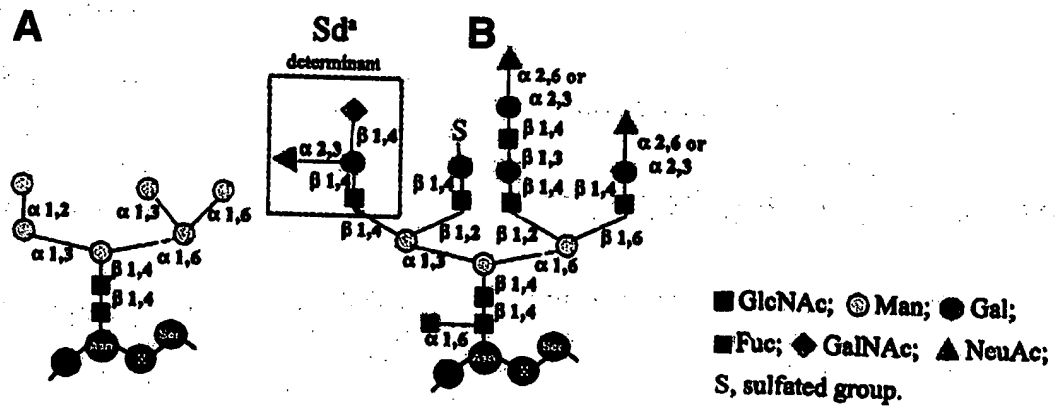

Fig 2. Representative structures of human THP *N*-glycans: (A) the major high-mannose sequence[47] and (B) the structure of a tetra-antennary chain showing the various nonreducing terminal units.[38,39]

age of the hydrophobic peptide, adds the preformed GPI anchor to the "new" C-terminus (Fig 1).[15] Rindler et al[16] transfected THP cDNA into HeLa cells and showed that THP is a GPI-anchored protein. Fatty acids bound to the GPI anchor allow insertion of THP into the outer leaflet of the plasma membrane (Fig 1). DNA encoding THP in the rat, mouse, and calf also was sequenced,[17-19] and the THP gene was reported present in all vertebrate classes.[20] The human THP gene was assigned to chromosome 16p12.3-16p13.11.[21] The mouse THP gene promoter was isolated, and by using the transgenic mouse approach, a sequence of 3.0 kb was shown to be sufficient to drive kidney expression of a heterologous reporter gene.[22]

On the basis of their amino acid sequence, a new class of GPI-anchored proteins, broadly homologous to THP, was identified. This class includes pancreatic glycoprotein 2 (GP2), which shows a 53% identity and 85% similarity to the human THP amino acid sequence[17,23]; a sea urchin sperm membrane protein[24]; and ZP2- and ZP3-glycoproteins, which participate in forming the transparent coat (zona pellucida) surrounding the eggs of all placental mammals.[25] GP2 is the major protein present in the zymogen secretory granule membrane of the panereas and very likely is involved in the regulated release of zymogens.[26] Recently, Jovine et al[27] showed that (1) the peptide encompassing amino acids 1 to 291 of human THP is selectively degraded by pancreatic elastase, and (2) the residual C-terminal peptide chain of approximately 48 kd that THP shares with ZP2- and ZP3-glycoproteins (ZP domain) is responsible for polymerization of all these glycoproteins (Fig 1).

The first electron microscope and ultracentrifuge studies attributed to urinary THP an axial ratio of 250 and a molecular weight of 7×10^6 M_r.[28,29] Subsequent studies showed that THP consists of protofilaments with a double-helix structure that, in polymer form, acquire a ribbon-shaped structure 1,500 to 4,000 nm in length and are organized in a large network.[5,27] In SDS-PAGE, THP shows an apparent molecular weight of 80 kd in nonreducing conditions or 97 kd when the glycoprotein is reduced with 2-mercaptoethanol.[7,30] The decrease in apparent molecular weight in nonreducing conditions depends on a high degree of polypeptide constraint imposed by the large number of intrachain disulfide bridges, in that all cysteine residues are converted into intrachain disulfide bonds in urinary THP.[31]

Compared with the apparent molecular weight of human urinary THP, the renal GPI-anchored counterpart has a greater value that does not change under GPI-specific phospholipase treatment, indicating that proteolytic cleavage in the juxtamembrane region of the THP ectodomain is responsible for the urinary excretion that occurs.[30] Consistent with this, recombinant THP expressed by HeLa cells is released mainly devoid of ethanolamine, the residue responsible for binding the GPI anchor to the THP C-terminus[32] (Fig 1). According to Fukuoka and Kobayashi,[33] proteolytic cleavage occurs between amino acids 524 (phenylalanine) and 525 (serine), ie, 66 amino acids upstream of the putative C-terminal residue bound to the GPI anchor.

THE GLYCOMOIETY STRUCTURE OF THP

Carbohydrates account for approximately 30% of the weight of human THP and consist mainly of *N*-linked glycans of di-, tri-, and tetra-antennary type[6,34-36]; however, 1 *N*-glycosylation site carries high mannose sequences.[37] The tetra-antennary chains are elongated in a sizable percentage by repeating *N*-acetyllactosamine sequences and show marked heterogeneity because of differences in the extent of sialylation, fucosylation, and sulfation (Fig 2).[38,39] Of the 8 potential *N*-glycosylation sites predicted by amino-acid sequence, only 7 are actually glycosylated.[40]

In addition, *N*-acetylgalactosamine (GalNAc) in β1,4 linkage to galactose occurs in α2,3 sialylated glycans, producing the GalNAcβ1, 4(NeuAcα2,3)Galβ1,4GlcNAc-sequence known as Sd^a antigen (Fig 2).[41] This antigen was originally described as a blood group determinant inherited as a dominant characteristic present in approximately 90% of the Caucasian population.[42] The GalNAc residue is the immunodominant sugar, confirmed because THP from Sd^a-positive individuals contains 1% to 2% of GalNAc, whereas THP from Sd^a-negative individuals does not contain GalNAc.[43] Biosynthesis of Sd^a determinant depends on a specific β-GalNAc-transferase (Sd^a-βGalNAc-transferase) first described in our laboratory.[44,45] Sd^a-βGalNAc-transferase activity is detected easily in the outer

medulla of human kidney, but not in the cortex, suggesting that the Sd^a antigen is carried prevalently by THP synthesized by cells from the thick ascending limb (TAL) of Henle's loop, rather than those from the early region of convoluted distal tubules.[46]

In human THP, high-mannose glycans are carried by Asn_{251},[40] and $Man_6GlcNAc_2$ is the preponderant structure over $Man_7GlcNAc_2$ and $Man_5GlcNAc_2$.[37,47] Recombinant THP expressed by transfected HeLa cells also bears high-mannose sequences similar to those found in urinary THP, indicating that the occurrence of such a structure is host-cell independent, ie, it is imposed by the protein primary structure of THP.[48] The percentage of distribution of high-mannose sequences in calf THP is very similar to that of human THP,[49] whereas pig THP has a much greater molar percentage of $Man_5GlcNAc_2$ (47% of total high-mannose sequences in pig THP versus 8% in human THP; Cavallone et al, manuscript submitted). The difference in the branching chain of high-mannose glycans between pig and human THP might affect the interaction with type 1 fimbriated *Escherichia coli*.[50] Recently, *O*-linked chains also have been described as present in THP.[51]

A question that has given rise to much controversy is whether the carbohydrate moiety of THP from pregnant and nonpregnant women differs.[49,51-54] An accurate recent analysis[55] established that THP from pregnant women (also termed uromodulin) shows a small increase in $Man_7GlcNAc_2$ molar percentage ($Man_7GlcNAc_2$ represents 35% of total high-mannose glycans in pregnant women versus 30% in nonpregnant women). Conversely, neither in the course of pregnancy nor 1 month after gestation was a significant change observed in the negative charge distribution of complex-type glycans, indicating no change in the content of sialic acid and sulfate residues.

LOCALIZATION AND BIOSYNTHESIS OF THP

When RNAs isolated from approximately 150 different cell tissues were hybridized using a large probe for human THP RNA, only RNA from human adult kidney gave a positive signal, indicating that this glycoprotein is exclusively produced by kidney cells.[13] Immunofluorescence and immunochemical analyses by light and electron microscopy indicated that THP resides in kidney cells of TAL and early distal convoluted tubules.[56-61] When the cellular location of rat THP messenger RNA (mRNA) was investigated by in situ hybridization using a radiolabeled human THP complementary RNA antisense probe (>600 bp), a positive reaction was found along the entire length of the TAL, but not in the macula densa or distal convoluted tubules.[62] This last observation is in agreement with previous immunofluorescence analyses of hamster and human kidney performed in the laboratory of Robin D. Marshall,[59,60] indicating that macula densa cells lack THP. Gokhale et al[63] used an RNA probe specific for rat THP and observed a strongly positive mRNA signal in the TAL region alone, even in conditions in which rat THP was detectable in papillary tubules. This result strongly supports the idea that rat THP synthesis is restricted to the TAL of Henle's loop. In a Western blot, we detected a relative distribution of human THP in kidney lysates of the outer medulla approximately 4-fold greater than in the cortex.[46]

The first ultrastructural studies by Hoyer et al[57] and Seiler and Hoyer[58] failed to show in rats that THP is distributed preferentially at the luminal face of TAL cells. Subsequently, Bachmann et al,[61] using protein-gold immunocytochemistry, observed a prevalent localization of rat THP in vesicles in close spatial relationship to the Golgi cisternae, fused with the apical face of TAL cells, and visualized slight positive gold labeling on the basolateral face. Conversely, various experimental approaches support the notion that THP is delivered to the luminal plasma membrane: (1) the GPI anchor and *N*-linked glycans both act as signals for vectorial transport of glycoproteins to the apical surface of epithelial cells,[64,65] (2) prevalent exposure of THP at the luminal cell surface is consistent with both substantial excretion in luminal fluid and scarcity in blood,[66] and (3) recombinant THP expressed by Madin Darby canine kidney (MDCK) cells is delivered entirely to the apical face and released in the corresponding medium.[67]

THP has been found in kidney distal tubules of one 14-week human fetus and in fetal rats,[68] whereas that found in human amniotic fluid very likely is derived from the fetus.[69]

The biosynthesis of recombinant THP ex-

pressed by HeLa cells shows that THP accumulates intracellularly as a precursor of approximately 86 kd, which is converted into the mature glycoprotein with an apparent molecular weight of 97 kd (in reducing conditions). This mass shift depends on the processing of most high-mannose glycans to polyantennary species.[48] Two bands with a difference in migration similar to that of precursor and mature forms of recombinant THP have been visualized by Western blotting in lysates from human and rat kidney.[30,70] When HeLa cells exspressing THP are treated with mannosamine, an inhibitor of GPI anchor biosynthesis, THP accumulates intracellularly and is significantly less exposed at the cell surface, indicating that GPI anchor addition is required for delivery to the plasma membrane.[32] Figure 3 shows the routing of GPI-anchored THP to the cell surface.

Both the first radioimmunoassay[8] and the latest enzyme-linked immunosorbent assay (ELISA) method[71] have shown that on consecutive days, there is a large variation in daily THP excretion under physiological conditions. Various parameters, such as urine volume and excretion of calcium, sodium, potassium, and creatinine, have been evaluated in relationship to daily THP urinary release, as have physical exercise, body weight, and diet, but controversial results have been obtained.[71-74] Moreover, the half-life for THP turnover in a single individual was reported to vary from a minimum of 3 to a maximum of 168 hours.[74] Recently, a significant increment in renal expression of THP in rats given a high-salt diet was observed.[75] Because THP urinary excretion is a multistep process that includes biosynthesis rate, complex posttranslational processing, and proteolytic release from the GPI-anchored counterpart, it is not surprising to find a broad scatter in the final step.

PROPERTIES OF THP

Tendency to Gelation/Aggregation

One of the most peculiar features of THP in solution is its tendency to gelation/aggregation when sodium chloride concentration is close to 100 mmol/L or calcium chloride is 1 mmol/L.[76,77] Both conditions usually occur in normal urine, and a method based on this property has been set up to purify THP. When urine of healthy individuals is filtered through a diatomaceous earth filter, THP is entrapped selectively in the filter. Because gelation is reversed at a low ionic concentration, THP is desorbed from the filter by deionized water and isolated to homogeneity by means of 2-step filtration and washing.[78] Recently, the salt precipitation method originally described by Tamm and Horsfall was compared with the diatomaceous earth filter method for isolating THP from urine of proteinuric patients and pregnant women, and better purification was obtained using the latter method.[79]

Interaction of THP With Other Proteins

Many reports indicate that THP binds to proteins present in urine, particularly those occurring in pathological conditions, but the tendency to gelation/aggregation of THP at a sodium chloride concentration close to 0.1 mol/L might have been responsible for overestimation of the binding property of THP. A clear interaction between immunoglobulin G (IgG) and urinary THP was observed by Rhodes et al.[80,81] They used a Scatchard plot to analyze the interaction of a monomeric form of THP, obtained by urea solubilization, to avoid the salt-dependent insolubilization of polymeric THP. In their first article,[80] these researchers reported a very high-affinity association between THP and IgG with a dissociation constant (K_D) ranging from 10^{-13} to 10^{-10} mol/L. In the second report, the same investigators corrected the K_D value, which actually ranged from 10^{-9} to 10^{-7} mol/L, but confirmed the biological significance of the interaction.[81]

Differences in the binding of IgG to THP have been observed in patients with glomerulonephritis or interstitial nephritis and related to changes in the carbohydrate moiety of THP, whereas in children with lymphoid cell malignancies, the alteration in THP glycomoiety does not affect interaction with IgG.[82,83]

IgG light chains also bind THP, and when they are largely present in urine, as in patients with myeloma, this interaction is responsible for the cast nephropathy accompanying this disease[84] (see Cast Nephropathy). Both C1q and C1 bind THP with an affinity that decreases significantly when the electrolyte concentration of the THP solution shifts from 0.020 to 0.15 mol/L of sodium chloride.[85,86]

Owing to the heterogeneity of the large THP glycomoiety, there are many observations about

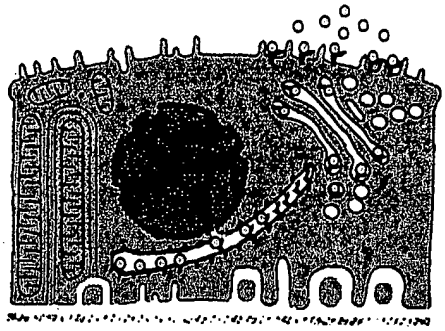

Fig 3. Schematic diagram of the biosynthesis and intracellular routing of GPI-anchored THP in a TAL cell. The addition of preformed GPI anchor to THP occurs in the ER: the membrane-bound form is transported to the Golgi complex, where glycans are fully processed, delivered to the luminal cell surface, and released in urine by a proteolytic cleavage (Fig 1). Note the large mitochondria and interdigitations in the basolateral membrane that are prominent in TAL cells.

its capacity for binding plant lectins, particularly those used as mitogens for lymphocyte blastogenesis, such as phytohemagglutinin (PHA), pokeweed mitogen, and concanavalin A.[11,87] The strong inhibitory effect of urinary THP on PHA-induced lymphocyte blastogenesis is caused by the high-affinity interaction between PHA leucoagglutinin-subunits and tetra-antennary glycans, which are largely carried by THP.[88-90] Very likely, the immunosuppressive activity assigned to THP from pregnant women (also termed uromodulin) depended on the competitive interaction of THP with PHA or pokeweed mitogen used in stimulating lymphocyte proliferation.[10,14] Subsequent reports by the group of Muchmore[52,91] showed that THP glycomoiety was entirely responsible for both in vitro inhibition of the antigen-specific lymphocyte proliferation and binding to recombinant interleukin-1 (IL-1) and recombinant tumor necrosis factor (TNF). According to Moonen and Williamson,[92] soluble native cytokines do not bind to THP.

In our laboratory, we have shown that when high-mannose or complex-type THP glycopeptides are covalently bound to albumin, the neoglycoprotein inhibits the lymphocyte blastogenesis induced by 1-way mixed lymphocyte reaction. On this basis, we proposed that inhibition of lymphocyte proliferation is dependent on a multivalent interaction between THP glycans and ligand(s) at the lymphocyte surface, and this interaction competes with the carbohydrate recognition system between effector and stimulator cells, which in the mixed lymphocyte reaction, mediates the blastogenesis.[93] Recently, it was reported that specific glycans of THP isolated from healthy adult males interact with IL-1β, and glycans containing either *N*-acetylgalactosamine or sulfate residues in specific isomeric linkages are responsible for the inhibition of lymphocyte proliferation induced by tetanus toxoid.[94]

Binding to and Activation of Leukocytes by THP

Several reports have shown that urinary THP binds to and activates leukocytes, including poly-

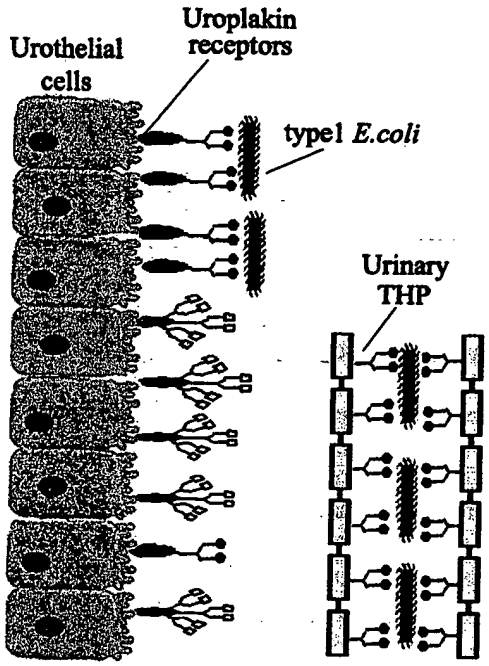

Fig 4. Binding of polymeric THP to type 1 *E coli*, mediated by high-mannose glycans, competes with adhesion of pathogens to uroplakin receptors. Uroplakins are the major integral-membrane glycoproteins exposed at the luminal face of the bladder and urothelial tract. In the drawing of THP, polyantennary glycans have been omitted.

morphonuclear neutrophils (PMNs), lymphocytes, and monocytes. PMN activation is mediated by their interaction with THP (in monomeric form) through a single class of sialic acid–specific receptors exposed in great number at the surface of PMNs.[95,96] Integrins also have been proposed to mediate the interaction between PMNs and THP through arginine-glycine-aspartic acid (RGD)-sequence mapping at position 142 to 144 of THP.[97] Recently, it was shown that recombinant THP produced by MDCK cells also binds to and activates PMNs.[67] According to Yu et al,[98] THP from pregnant women increases the phagocytic activity of PMNs and prostaglandin E_2 release, suggesting that a specific interaction occurs between THP and the membrane of PMNs. Similarly, blood mononuclear cells are activated by THP from pregnant women;[99] in this case, enhanced release of IL-1, IL-6, and TNF from monocytes very likely is responsible for the proliferation of both B and T lymphocytes. In a more recent study, Su et al[100] observed that THP from nonpregnant women also induces the secretion of TNF and expression of tissue factor by resting blood monocytes; however, both effects were several fold lower than those induced by THP from pregnant women (uromodulin).

Human monocytes isolated from peripheral blood and deprived of lymphocytes are able to phagocytize a particulate form of THP prepared from urine of healthy individuals and generate reactive oxygen metabolites, as well as release 95-kd gelatinase and other lysosomal enzymes.[101] It is worth noting that the protection of mice against a lethal inoculum of *Listeria monocytogenes*, previously assigned to THP by Fontan et al,[102] must be assigned to a minor protein (HGP92) also precipitated from normal urine by 0.58 mol/L of sodium chloride that shows an apparent molecular weight of 92 kd, very close to that of THP.[103]

Binding of THP to Uropathogenic Strains of E coli

An interesting line of research concerns the role of THP in the kidney defense against urinary tract infections (UTIs), particularly those caused by *E coli*.[104-107] UTIs occur in a large number of individuals (particularly women and children in developed countries), and the majority are caused by *E coli*.[108] Colonization is mediated by the binding of lectin-like adhesins present on *E coli* fimbriae to carbohydrate structures carried by glycoproteins and glycolipids exposed at the cell surface. *E coli* fimbriae are classified according to their sugar specificity: type 1, type P, and type S recognize the high-mannose glycan, Galα1,4Galβ-sequence present in globoseries of glycolipids and the NeuAcα2,3Gal sequence of sialylated glycans, respectively.[109] Urinary THP carries both high-mannose and NeuAcα2, 3Gal sequences and thus may be considered a ligand for both type 1 and type S fimbriated *E coli*. There is evidence that type 1 *E coli* strains represent the predominant phenotypic variants of isolates from patients with UTIs,[110] and uroplakin Ia and Ib (the most abundant integral membrane glycoproteins of the luminal surface in urothelial cells) behave as efficient cell receptors for type 1 fimbriated *E coli*.[111-114] Pak et al[107] recently showed that THP binds type 1 fimbriated *E coli* in vitro and efficiently competes with uroplakin Ia and Ib in binding to these pathogens. These results support the notion that in vivo, urinary THP represents a protective agent against UTI. Figure 4 shows the putative role of urinary THP in protection against UTIs.

CLINICAL RELEVANCE OF URINARY THP

Urinary THP is believed to be involved in the pathogenesis of various disorders of distal nephrons and the urinary tract, such as cast nephropathy, urolithiasis, and tubulointerstitial nephritis (TIN). Moreover, reduced urinary THP excretion is considered a reliable index of distal tubular cell damage. Finally, a very recent study showed that familial juvenile hyperuricemic nephropathy (FJHN) and autosomal dominant medullary cystic kidney disease 2 (MCKD2) arise from mutations of the THP gene.[115]

Cast Nephropathy

Microscopic observation of casts allows one to distinguish casts according to size and morphological characteristics. Although size reflects the shape of the tubules in which casts are formed, eg, they appear convoluted when formed in the convolute part of a distal nephron, a classification based on morphological characteristics consists of hyaline, granular, fatty, and leukocyte casts. THP is by far the predominant protein of hyaline casts, but also is present in the matrix of

casts in which plasma proteins or hemoglobin are abundant, as well as in granular casts containing debris from damaged kidney cells or blood cells.[116]

McQueen[117] first proposed that THP was present in urinary casts. Subsequently, using a fluorescence antibody technique, he presented direct evidence of the widescale occurrence of THP in the matrix of urinary casts.[118] Interestingly, in his first article, McQueen[117] also reported that myeloma protein precipitates THP in aqueous solution. A few years later, Fletcher et al[119] showed that THP isolated from casts of 4 patients with nephrotic syndrome and urine of healthy individuals showed a very similar amino-acid composition and carbohydrate content. More recently, Fairley et al[120] examined the protein composition of urinary casts from 60 patients with glomerulonephritis and 46 healthy individuals subjected to strenuous exercise. In addition to THP, plasma proteins, particularly IgA, IgG, or IgM, were found in the cast matrix of all patients, but THP was the only protein detected in hyaline casts from 41 healthy individuals, whereas in the remaining 5 individuals, fibrin, C3, and C1q, but not immunoglobulins, were present.

Recently, Fogazzi and Testanera[121] reported that on the basis of histological examinations and chemical analyses of kidney and urinary casts, Carlo R. Rovida, who ran the Institute of Clinical Medicine at the University of Turin (Italy) from 1874 to 1877, first described a specific protein, termed cilindrina, produced by kidney tubular cells as the major constituent of hyaline casts.

The involvement of THP in hyaline cast formation is facilitated by its tendency to gelation/aggregation at a salt concentration close to isosmolarity.[76] It is worth noting that in the TAL, the tubular fluid in physiological conditions is at a low electrolyte concentration in that epithelial cells are not permeable to water, whereas chloride, sodium, and potassium are actively absorbed through Na,K-adenosine triphosphatase activity. Thus, in physiological tubular fluid in which THP is released from the GPI-anchored counterpart, the electrolyte concentration disfavors the gelation/aggregation of THP. There is evidence that removal of all *N*-linked glycans by *N*-glycanase treatment negatively affects the gel-forming tendency of THP.[122,123]

The presence in urine of plasma proteins, particularly IgG and IgG light chains, is a crucial condition for cast formation. We observed that when IgG is excreted heavily by proteinuric patients, it binds to THP covalently linked to Sepharose in an amount that decreases significantly when sodium chloride concentration is increased from 0.02 to 0.15 mol/L. Figure 5 shows results of urine analysis from a patient with nephrotic syndrome (proteinuria, 3.7 g/L of protein). Similar results were obtained when proteinuric patients with other nephropathies were analyzed. These results support the notion that the electrolyte concentration of tubular fluid has a part in cast formation, particularly when IgG is present in urine.

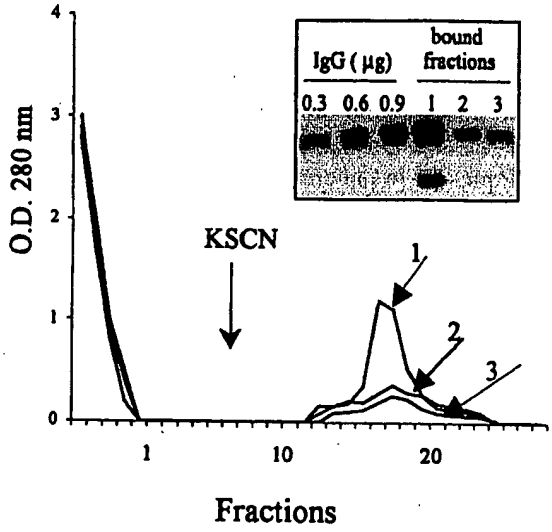

Fig 5. Representative profiles of affinity chromatography on a THP-CNBr-Sepharose column of urine from a patient with nephrotic syndrome (proteinuria, 3.7 g/L of protein). Arrows 1, 2, and 3 indicate the chromatography profile of urine dialyzed against 0.02, 0.08, and 0.15 mol/L of sodium chloride, respectively. (Insert) Western blot of pooled fractions containing bound proteins eluted by 3 mol/L of KSCN; lanes 1, 2, and 3 correspond to the 1, 2, and 3 chromatography. SDS-PAGE was performed in nonreducing conditions, and the blot was developed using anti-IgG antibodies.

Many studies[84,124-127] reported the interaction between IgG light chains and THP. The THP sequence encompassing amino acids 225 to 233 appears to be crucial for binding to IgG light chains,[126] and the third complementary-determining region of both κ and λ light chains is required for binding to THP.[127] Leboulleux et al,[128] using an ELISA procedure, found that 5 of 12 samples of light chains from patients with cast nephropa-

thy did not react with THP and suggested that both a low affinity of certain light chains and differences in cast pathogenesis may explain these results. Consistent with the implication of THP in cast formation, GP2, a homologue of THP present in pancreas cells, is the major component of intraductal plugs of pancreatic juice.[129]

Urolithiasis (Stone Disease)

Although the water-conservation function of the kidney produces supersaturation of various insoluble salts, such as calcium oxalate, calcium phosphate, and urate salts, only approximately 3% of the adult population of the Western hemisphere has nephrolithiasis.[130] The relatively low incidence of this disease depends essentially on 2 conditions: the presence in tubular fluid and urine of stone-formation inhibitors and the complex process required for the growth of microscopic crystal to a size producing stone disease. Stone formation usually takes place in various phases: the first consists of nucleation of supersaturated salts. Nucleation also may occur on heterogeneous surfaces present in tubular fluid or urine, eg, cell debris or urinary casts; subsequently, the microscopic nuclei undergo aggregation and grow into macroscopic stones.[130] Adherence of crystal aggregates to the tubular cell surface also is considered to have an important role in stone formation.[131-133]

THP was found in human kidney stones in 1965 by Keutel,[134] and in 1973, Grant et al,[135] using a radioimmunoassay method, showed that THP content ranges from 0.002 to 5.07 mg/g of renal and bladder calculi, and there is no correlation between amount of THP and qualitative composition of inorganic components of stones.

The involvement of urinary THP in crystal nucleation has not been consistently proved,[136] whereas in an in vivo rat model, THP does not appear to mediate the initiation of crystal formation.[137] The inhibitory effect of THP in crystal aggregation and growth has been described in the case of both calcium oxalate[138-140] and hydroxyapatite stones.[141] There is consensus that inhibition of crystal growth in normal urine is caused mainly by urinary macromolecules, rather than low-molecular-weight components, and this property has been associated with the polyanionic structure of the major inhibitors.[142] For instance, heparin and chondroitin sulfate are rich in SO_4^{--} groups,[143] whereas nephrocalcin, osteopontin (uropontin), and F1 activation peptide of prothrombin belong to the aspartic acid–rich protein superfamily.[144-146] THP also is a polyanionic macromolecule (3.5 isoelectric point) because of the large extent of sialylation (18 residues/molecule according to Dustan et al[9]) and the presence of sulfate groups bound to *N*-linked glycans.[38] Recently, the presence in the luminal face of tubular cells of sialylated glycans carried by glycoproteins and glycolipids has been seen as crucial in the nucleation of calcium oxalate dihydrate crystals.[147] Conversely, adhesion of uric acid crystals to renal cells in culture (MDCK and BSC-1 line) was suggested to be mediated mainly by hydrogen bonds and hydrophobic interactions.[148] Because GPI-anchored THP is one of the main sialylated glycoproteins exposed at the luminal face of the first tract of distal nephrons, it should be involved in the calcium oxalate nephrolithiasis process even before it is released in urinary fluid. A recent study[149] reported decreased THP expression in the kidney of rats treated for 8 weeks with ethylene glycol to obtain calcium oxalate urolithiasis. However, reduced expression was detected only after 4 weeks of treatment, when crystal aggregates of calcium oxalate were visualized in the kidney of treated rats.

The difference in degree of sialylation between urinary THP from healthy individuals and recurrent calcium oxalate renal stone formers has been investigated extensively. Some studies[150-153] reported an increase in isoelectric point of THP from recurrent calcium oxalate stone formers, probably dependent on a reduced sialic acid content. However, this observation was not confirmed subsequently.[63,154] Despite this, partial removal of sialic acid from THP by neuraminidase treatment results in loss of in vitro inhibition of urinary crystal aggregation,[155,156] consistent with the notion that polyanionic structure is crucial for inhibition of calcium oxalate crystal aggregation.

The involvement of urinary macromolecules in the modulation of crystal aggregation, as well as their participation in stone formation, was analyzed in some studies by using diluted urine or urine processed by centrifugation or filtration. The last 2 procedures, particularly filtration, significantly reduced THP content in processed

urine.[157] It is evident that this behavior may have been responsible for discrepancies in results concerning the role of THP as a modulator of urolithiasis, particularly when this role was compared with that of other urinary modulators that do not significantly change concentration on urine processing.

TIN

Bacterial infections, vesicouretereal reflux, exposure to heavy metal, and antibiotic or analgesic intake are the major etiologic agents of TIN, which is characterized in the first phase by infiltration of inflammatory cells (mononuclear cells, lymphocytes, plasma cells, and PMNs) into kidney interstitium and subsequently by interstitial fibrosis and tubular atrophy.[158] Clinical studies indicated that renal diseases, namely, chronic interstitial nephritis, medullary cystic disease, reflux nephropathy, and rejecting renal allografts, very often are accompanied by abnormal THP deposits in the tubular interstitium, and there is an immunologic response to THP.[159-163] THP is a powerful autoantigen, and immune deposits containing THP and anti-THP antibodies have been localized in the intercellular space of TAL in rats, mice, and rabbits challenged with homologous THP.[58,164-167] In all likelihood, healthy mammals, including humans, do not produce anti-THP antibodies because the exclusive localization of THP at the luminal face of tubular cells segregates the protein from the immune system. Segregation may be abolished by various alterations to cells expressing THP, such as loss of their apical/basolateral polarity and the consequent release of THP from the wrong side, as well as conditions altering cell integrity. Nonvectorial delivery of THP to the luminal surface also may occur in conditions that alter the routing of THP along the exocytic pathway. Malagolini et al[32] observed that under treatment with monensin, an ionophore that interferes with the function of the Golgi apparatus, a THP that is not fully glycosylated and not exposed at the cell surface is released from cells expressing THP. Because the GPI anchor is a sorting signal to the cell apical face,[64] if the GPI anchor is lost along the exocytic pathway, THP also might be released from the basolateral face and enter the peritubular capillaries; tubular basement membranes are highly permeable to macromolecules.[168]

Several studies have shown that interstitial deposits of THP and THP-immune complexes frequently are surrounded by neutrophils, mononuclear cells, and plasma cells.[159-164] Although binding of THP to neutrophils may be responsible for the acute inflammatory response,[95-97] binding to mononuclear cells may extend the reaction to the chronic phase characterized by fibrosis.[101,165] Although the proinflammatory activity of THP has been put forward as involved in TIN pathogenesis,[96,101] both clinical and experimental observations indicate that abnormal THP deposits in the kidney do not have a crucial role in inflammatory processes. For instance, on the basis of 124 renal biopsies of patients with various nephropathies, Chambers et al[169] concluded there is no correlation between THP accumulation in the interstitium and tubulointerstitial damage, although in the majority of patients examined, THP antibodies were present. The absent or low inflammatory response to extratubular THP deposits (particularly PMN infiltration) also was reported in renal allografts and patients with urinary extravasation.[170-172] Again, in experimental models of unilateral ureteral obstruction in rats or mice, as used by Dziukas et al[173] and Fasth et al,[174] THP accumulation in the interstitium is not accompanied by inflammatory response. These investigators suggested that in urine extravasation, toxin components other than THP may be responsible for the inflammation and TIN pathogenesis.

When transfected HeLa cells stably expressing cell surface–anchored THP were incubated with human neutrophils, no binding was found, but opsonization of cells with anti-THP antibodies resulted in (1) dramatic adhesion and myeloperoxidase activation of PMNs, (2) a significant increase in THP release, and (3) exit of the partially processed THP from the cells. These results support the notion that after the autoimmune response, cell surface–anchored THP also may contribute to the pathogenesis of TIN.[175]

Involvement of THP in tubular cell damage also has been related to its ability to bind to C1q, the component of the complement pathway responsible for initiating the complement cascade.[85,86] There is evidence that in some renal diseases (eg, nephrotic syndrome), complement

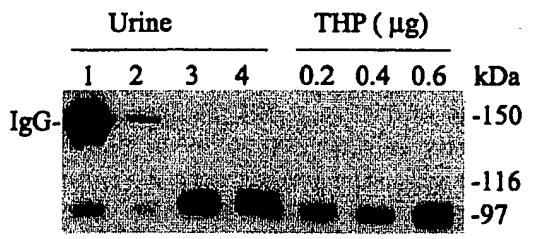

Fig 6. Western blot of urine samples from patients with lupus nephritis (lane 1) and MCKD (lane 2) and 2 healthy individuals (lanes 3 and 4). An identical percentage of daily urinary volume (0.5%) was applied in each lane. Urinary THP used as a marker was isolated from pooled urine samples of healthy individuals. SDS-PAGE was performed in nonreducing conditions, and the blot was developed using both anti-THP antibodies and anti-IgG antibodies. As expected, the latter antibody visualized a large presence and a trace of IgG in urine from patients with lupus nephritis and MCKD, respectively, and absence of IgG in healthy individuals.

factors are excreted copiously and activated in urine.[176] Thus, either complement per se or the THP-C1q complex may accumulate in the interstitium and have an important role in TIN pathogenesis.

THP Excretion in Nephropathies

Abnormalities in daily urinary excretion of THP have been detected in several pathological states of the kidney and urinary tract, and decreased excretion is considered a reliable index of damage to the TAL cells that synthesize THP.[73,177,178] Our laboratory detected a marked reduction in daily THP excretion in a patient with medullary polycystic kidney disease (Fig 6). In active lupus nephritis, decreased THP excretion was related to impairment of the distal nephron function that accompanies the active phase of the disease[179] (Fig 6). Again, marked reduction in urine THP from infants with hyperprostaglandin E syndrome was related to a congenital defect of the distal nephron.[180] THP excretion also is reduced in urine of stone formers, very likely because stones per se damage the tubular epithelium of the TAL.[181-183] Interestingly, a study[184] of THP excretion after uninephrectomy in kidney donors has shown a persistent increase (~40%) in THP excretion from the kidney that remains in the donor and good correlation between THP excretion rate and glomerular filtration rate.

Urinary THP has been studied in diabetes mellitus, particularly the insulin-dependent type.[185-193] A reduction in THP excretion was observed consistently when diabetic nephropathy is characterized by a reduction in renal mass, which usually occurs at a late stage of disease.[185-190] Conversely, other investigators observed increased excretion in the early stage of the disease, as well as during acute euglicemia and water load.[191-193] In experimental diabetes induced in rats by streptozotocin, THP excretion was increased markedly, an effect supposed to be associated with distal tubular dysfunction.[194,195] According to Agardh et al,[195] greater excretion is attenuated by compounds that inhibit both the production of advanced glycation end products and oxidative stress, 2 processes involved in the development of diabetic nephropathy.

Mutations to the THP Gene in FJHN and MCKD2

The autosomal dominant MCKD2 and FJHN, very likely 2 facets of the same disease, are characterized by hyperuricemia, medullary cysts, interstitial nephritis, and progressive renal failure.[196] By genome-wide linkage mapping in Italian, Czech, and Belgian families, new loci for MCKD2 and FJHN were identified in the regions of chromosome 16p11.2 and 16p12,[196-198] close to the localization of the human THP gene.[21] Mutations of the THP gene were identified very recently by Hart et al[115] in several individuals from 3 unrelated families with FJHN and 1 family with MCKD2. The missense mutations identified consist of the deletion or addition of 1 cysteine residue and 1 frame deletion of 9 amino acids. These results provide the first demonstration that FJHN and MCKD2 arise from allelic mutations of the THP gene and suggest that an alteration to the tertiary structure of THP, particularly the misfolding caused by the uncoupling of at least 1 Cys-Cys bond, is responsible for the disease(s).

We observed[32] that when HeLa cells expressing THP are treated with an exogenous reducing agent, such as 2-mercaptoethanol, routing along the exocytic pathway of partially reduced THP is delayed considerably, indicating that completion of intrachain disulfide bonds is required for a regular exit from the ER and delivery of THP to the cell surface. On this basis, one may postulate that misfolded THP produced in MCKD2 and FJHN undergoes a similar fate, and accumula-

tion in the ER may result in altered biosynthesis, eg, impairment of the GPI addition and glycosylation processing, 2 events crucial for the delivery of THP to the luminal surface of the tubular epithelium. These effects may explain the TIN associated with the disease(s).

THP IN BIOTECHNOLOGY

Observations that the THP gene is transcribed exclusively by TAL cells and THP is largely excreted in urine recently have been used for the transgenic production of human therapeutic proteins.[199,200] This approach is based on recently developed technologies that allow one to generate transgenic mice by using a mouse gene promoter to direct the expression of proteins selectively released in urine.[201] In comparison to the expression of human therapeutic proteins in milk of transgenic livestock, production by kidney cells and the consequent release in urine appears to offer a much more cost-effective advantage in that urine contains few proteins or lipids, a condition that facilitates isolation of the recombinant protein to homogeneity. In this way, recombinant human α_1-antitrypsin was isolated from transgenic mice. It shows a broad similarity in its activity to that produced by human hepatocytes.[199] When the same approach was used to produce human recombinant erythropoietin, its expression and excretion in transgenic mice was accompanied by disease symptoms similar to polycythemia in humans, confirming that the THP promoter is effective in directing the production of proteins capable of exerting an in vivo effect.[200]

Recently, research in the field of genomic manipulation acquired a new strategy, referred to as the Cre/loxP site-directed recombinant system.[202] This strategy provides an advantageous tool for spatially and temporally modulated somatic mutations. When stable transgenic mice expressing Cre recombinase, under a tissue-specific promoter, are mated with mice harboring the loxP DNA sequence, a new mouse line may be obtained in which the gene disruption mediated by Cre recombinase occurs only in cells in which the promoter is active.[202] Striklett et al[203] used this strategy and, using a THP promoter, found that Cre-recombinase is specifically expressed and active in cells of TAL. As these investigators emphasized, this approach introduces a new tool in specifically exploring the function of the TAL, which represents one of several regions of the kidney that variously contribute to renal physiological states.

CONCLUSION

Although the bulk of evidence shows the involvement of THP in pathological states of the kidney and urinary tract, there are no clear indications about its physiological role, although the structural, genetic, and cytological characterization largely has been clarified in the last 20 years. In the late 1980s, great enthusiasm accompanied the observation that urinary THP/uromodulin could have an immunoregulatory function in that it inhibits in vitro lymphocyte blastogenesis and interacts with cytokines.[204] Some considerations may be proposed to explain why this line of research has not confirmed initial expectations and hopes for the therapeutic use of THP. First, results obtained in vitro have not been carefully evaluated with respect to their in vivo relevance: in physiological conditions, THP is expressed exclusively by TAL cells, delivered to the luminal surface, and excreted in urinary fluid. Thus, it is improbable that a protein present in a cell compartment and fluid, where very few cells mediating the immune response occur, may show an immunoregulatory function.

The second consideration concerns the demonstration that the glycomoiety of THP is entirely responsible for inhibiting lymphocyte proliferation and cytokine binding.[52,91] Because completion of the carbohydrate moiety of a glycoprotein is dictated specifically by enzymes (glycosidases and glycosyltransferases) from cells responsible for expressing it,[205] large-scale production of a functionally active THP cannot be made by the usual cost-effective biotechnological techniques. Last, but not least, the property by which THP behaves as an efficient autoantibody prevents it from being used in vivo.

Considering that release of THP in the urinary fluid from the cell-surface GPI-anchored counterpart is relatively constant and abundant in physiological conditions and all mammals excrete THP, one may assume that its presence in urine is an advantage very likely imposed by selective pressure. Thus, the question is, what condition(s) served as a selective agent for this process? One possible answer is that urinary THP affords effi-

cient protection toward the most frequent infections of the urinary tract by binding uropathogenic strains of *E coli*.[107] The luminal cell surface of the respiratory and intestinal epithelium is covered by mucus, an effective agent in preventing adhesion of pathogens to glycoproteins and glycolipids exposed at the luminal plasma membrane. This protection is absent from the urinary tract in that no mucus (or only a very small amount) covers the luminal cells, particularly in the bladder, in which urine constantly resides. Because infections are caused mainly by *E coli* strains that enter the urinary tract by an ascending route from intestinal flora,[206] a real advantage seems to be offered by the presence of THP as a soluble ligand because, following the route and fate of urine, it may interact with the pathogens any time after their entry into the urinary tract and eventually be eliminated from the body. The occurrence of THP in urine as a large homopolymer also is a very advantageous property in that it behaves as a multivalent ligand, ie, it binds pathogens with a high affinity. In this context, addition of the GPI anchor also may be an advantage from an evolutionary point of view because the GPI anchor is a selective signal for delivering glycoproteins to the luminal cell surface of tubular epithelium and for the restricted release of THP into the urinary fluid.

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